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SEWAGE SLUDGE VIRAL AND PATHOGENIC AGENTS IN SOIL-PLANT-ANIMAL SYSTEMS

by

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16. ABSTRACT

A multidisciplinary study was carried out to determine the ultimate fate of various toxic elements or pathogens associated with Florida and Chicago municipal sludges when applied to soil-plant-water systems and to determine physiologic, pathologic, growth, and reproductive responses of cattle, swine, and foultry fed sludges, grains, or forages from soils pretreated with urban liquid digested sludges as well as health effects in mice receiving liver or kidney tissues from steers and swine exposed to such feeds or contaminants. Minimal differences occurred in growth performance or egg production in cattle, swine, or poultry fed forage or grain from soils pretreated with a variety of urban sewage sludges. Cattle and swine tissues, when fed to mice, resulted in alterations of the normal mineral balance as well as reproductive performance. Tissues from animals intended for human consumption exposed to sarcocyst contaminated sewage sludges may serve as health hazards for animals and humans. Application of urban sewage sludges at 19.8 t/hectare produced equivalent plant growth stimulation for corn, barley, wheat, and sorghum as commercial fertilizers. Certain bacteria, commonly associated with sludges, disappear in a few days after soil or plant application; however certain viruses and parasites were shown to persist.

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and governmental concern about the dangers of pollution to the health and welfare to the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our national environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to the valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

Recycling digested municipal sludges in agricultural systems is an attractive alternative method for their utilization if "safe" management techniques can be devised that do not adversely affect plant productivity or animal and human health. This study of the ultimate fate of various toxic elements and pathogens in sludges applied to soil-plant-animal systems will aid decision makers in selecting such techniques.

James B. Lucas
Acting Director
Health Effects Research Laboratory

ABSTRACT

This research was initiated to determine if digested sewage sludges could be applied to a soil-plant-animal system to improve soil fertility, increase forage and grain production, provide animal feeds necessary for optimal animal growth or performance without posing a hazard to plant and animal production or human health. The studies also included the persistence and movement of pathogens, drugs, or chemicals in soils, plant products, or animal tissues.

Beef steers were fed digested municipal sludges incorporated into feedlot diets and feeds (corn grain, forage, sorghum silages, and bahiagrass pastures) produced on land treated with sludge. These studies were conducted to determine the effects of these feeding programs on animal performance, carcass quality, and concentrations of selected toxic elements in liver, muscle, and kidney tissues. The performance and carcass data of treated steers in all of the studies were generally not different from the control steers.

Feeding sewage sludge on reproductive performance in female swine during successive gestation-lactation periods was evaluated. These studies indicate that breeding, farrowing, and rebreeding weights were reduced. Lactation and gestation weight changes were lower and fewer pigs were farrowed in sow groups fed 10 and 20% sewage sludge in their diets.

Duplicate experiments of 21 days duration were conducted with day-old broiler-type chicks and laying hens to study the influence of replacing one-half or all of the normal dietary corn complement with corn grown on soil fertilized with municipal sludge. Corn from the sludge-amended soil did not adversely affect final body weights or daily feed intake. Substitution of a sludge with high metal concentrations or equivalent levels of certain hazardous metals altered growth and laying performance.

Toxicity from feeding dried sewage sludge included in a normal swine starter ration, may occur from a deficiency of available protein or other essential nutrients, or from the accumulation of hazardous chemical residues. Cadmium exposure induced microcytic and hypochromic anemia. Cadmium also induced differences in the activity of liver serum enzymes in pigs exposed to aflatoxin B_1 or warfarin. This is the first demonstration of the cadmium blocking effect on the microsomal enzyme system in pigs. Of 7 pigs fed 10% Gainesville sludge, 4 had Sarcosporidia in the myocardium, and the hearts of 2 of the 4 pigs fed 20% contained the parasite. Among cattle fed Pensacola sludge, 19 of 32 contained Sarcosporidia in the cardiac muscle, while the cardiac muscle of 6 of 17 controls was parasitized. The presence of Sarcosporidia in hearts of swine and cattle fed sludge may be of public health significance.

Land spreading of sewage sludge is probably the most practical means of disposal for municipalities and cities. Uptake of certain metals by forage and grain crops from land treated with sludge may create health risks. Pre-1978 sludge from Chicago contained large quantities of copper, zinc, lead, and cadmium. The Pensacola sludge was high in zinc. Metal uptake of the corn plant was directly associated with soil pH. The higher the soil pH the smaller the quantity of metal uptake.

Sludge application at the 24 ton/ha rate compared favorably with mineral fertilizer as a source of plant nutrients for bermudagrass.

Samples of sludge, feed, feces, and animal tissues (kidney, liver, spleen, and blood were analyzed for pathogenic bacteria. Pathogenic bacteria were not found to be a significant hazard.

Viruses were not detected in topsoils 8 months after spreading Pensacola sludge. Enteroviruses represent a minimal hazard, either through translocation through grain or forage or with regard to groundwater contamination.

Samples of sludge, soil/sludge mixture, feed, and animal tissues (kidney, liver, fat, muscle) were analyzed for chlorinated hydrocarbon pesticide residues and also polychlorinated biphenyls. Little, if any, pesticide residues were present in sludges used in this research project.

This report was submitted in fulfillment of Grant No. R804570 by the University of Florida under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period September 1, 1976 through December 31, 1980 and work was completed December 31, 1980.

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DROPPED HEAD. BEGIN SECTIONS HERE

CONTENTS

Foreword iii
Abstract iv
Conclusions x
Recommendations xii
Acknowledgement xiii

EFFECTS OF LIQUID DIGESTED SLUDGE ON SOILS AND PLANTS 1

- The Nutrient Status of Six Field Crops Grown on Soils Treated with Liquid Digested Sludge 2
- The Utilization of Liquid Digested Sludge on Agricultural Land 5
- The Uptake of Certain Metals by Corn Grown on Soil Treated with Chicago or Pensacola Sewage Sludge 7
- Sludge Effects on Yield and Cadmium Uptake of Coastcross 1 Bermudagrass 8
- Transport of Sewage Sludge Constituents in a Sandy Soil and Uptake of Metals by Bermudagrass 15

CATTLE FEEDING TRIALS WITH SLUDGES OR FEED GROWN ON SLUDGE TREATED LAND 20

- Dried Pensacola Liquid Digested Sludge in the Diet of Steers 21
- Winter Annual Pastures Fertilized with Pensacola Liquid Digested Sludge and Grazed by Growing Beef Steers 27
- Dried Pensacola Liquid Digested Sludge (DPS) in the Diets of Feedlot Steers 30
- Dried Chicago Sludge and Corn from Soil Fertilized with Liquid Pensacola Sludge in Diets of Beef Steers 38
- Forage Sorghum Silages Grown on Soil Treated with Liquid Digested Sludge and Fed to Beef Steers 44

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BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS

PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 287)
TYPING GUIDE SHEET

CONTENTS (Continued)

Pensacola Bahiagrass Pastures Fertilized with Pensacola Liquid Sludge and Grazed by Beef Steers 56

CATTLE - OTHER HEALTH EFFECTS 71

The Effects of Recycled Cattle Manure on the Health and Performance of Steers 76

SWINE FEEDING TRIALS WITH SLUDGES 79

Performance and Tissue Minerals of Swine Fed Sewage Sludge Diets 80

Effect of Feeding Sewage Sludge Diets on Reproduction, Growth, and Tissue Mineral Accumulation 96

Effect of Feeding Digested Sewage Sludge on Long-term Sow Reproduction Performance 101

SWINE - OTHER HEALTH EFFECTS 113

Feeding Trials of Dried Urban Sludge and the Equivalent Cadmium Level in Swine 115

Toxicology of Aflatoxin B_1, Warfarin, and Cadmium in Young Pigs 127

High Performance Liquid Chromatographic Determination of Sulfonamides of Ionic Suppression 129

Comparison of the Pharmacokinetics of Sulfisoxazole in Humans and Two Monogastric Species 130

Comparison of the Potential Toxicity of Bilirubin in Humans and Two Monogastric Species after a Single Administration of Sulfisoxazole 131

POULTRY 132

Health Effects of Sewage Sludge and Grain from Sludge Treated Soils Versus Equivalent Metal Levels in Poultry . . . 132

HEALTH EFFECTS IN MICE FED DIETS THAT CONTAINED TISSUES FROM CATTLE OR SWINE FED SEWAGE SLUDGE RATIONS 149

MICROBIOLOGY 164

General Proceedings 164

Bacteriological Analysis of Feed, Grass, and Sludge 166

CONTENTS (Continued)

FATE OF VIRUSES FOLLOWING APPLICATION OF MUNICIPAL SLUDGE TO LAND 187

PESTICIDE RESIDUES IN FEED, SLUDGE, SOIL/SLUDGE, AND ANIMAL TISSUES 227

PUBLICATIONS 230

EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 287)

TYPING GUIDE SHEET

CONCLUSIONS

Land spreading of urban sewage sludge is probably the most practical means of disposal. Sewage sludge was shown equivalent or superior to commercial fertilizers for production of certain crops under Florida conditions. However, uptake of metals by forage and grain crops may create certain risks. Metal uptake by the corn plant was directly related to the soil pH; higher pH levels reduced their uptake. Levels present in grain were less than in the forage. Cadmium levels in forage from soils pretreated with certain sludges resulted in high levels in liver and kidney tissues of cattle consuming such forage. However, performance and carcass data of treated steers in these studies were not different from the data obtained with the control steers. Clinical chemistry tests and pathologic lesions suggested cumulative toxic effects including liver damage.

The 1979 steer trial, where animals grazed on forage from soils pretreated with Pensacola sludge and spraying of the sludge on the growing plants, resulted in presence of Sarcosporidia sp. in the cardiac and skeletal muscles. This may be of public health significance.

Incorporation of dried sewage sludge at ten to 20 percent of swine rations produced depressed weight gains and the 21 day weaning weights were lower in pigs from sows consuming the sludge-containing diets. The kidney cadmium levels of sows receiving the ten and 20 percent sludge levels were increased significantly, i.e., four ppm for controls and 17 and 24 ppm for the sludge rations; both lead and cadmium were increased in the liver and kidneys of weanling pigs. Reproductive performance was more suppresssed in the second generation sows than in the first.

Growth trials with Cobb broiler chicks compared the effects of poultry rations with 0, three, and six percent dried Chicago sludge. Increased levels of cadmium in the liver and kidneys occurred in those chicks receiving the increased levels of the sludge. However, none of the production criteria, i.e., production, daily feed intake, feed efficiency, egg weights, nor body weights, were adversely affected in Leghorn hens receiving such modified diets.

Having demonstrated that increased cadmium levels occurred in tissues from cattle and swine consuming feeds from sludge amended soils, these liver and kidney tissues were dried, ground, and incorporated into mouse diets. The finished diets contained a 15 percent level of protein and five percent levels of kidney and liver tissue. Metals were translocated through the cattle and swine tissues with increased levels of cadmium, nickel, chromium, and lead in liver and kidney tissues of mice. These increases in mice were associated

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with decreases in number of mice weaned in the treated versus the control groups.

Analysis for pesticide residues in the various sludges indicated that little, if any, chlorinated residues were present. It was concluded that these sludges presented no hazard from the aspect of pesticide residues.

Samples of sludge, feed, feces, and animal tissues (kidney, liver, spleen, and blood, were analyzed for pathogenic bacteria. Contamination was a major problem, both when collecting specimens during the trials as well as at slaughter. No enteric pathogens or *Mycobacteria* were isolated from these samples. There was one isolation of *Staphylococcus aureus*, and two isolations of *Streptococcus pyogenes* during the cattle and swine trials. Two group B *Salmonella enteritides* isolates were obtained from the feces of animals on a sludge amended diet plus three isolates at a later date from the same group. The very few positive isolates suggested that these three types of digested sewage sludges posed no significant health hazards from bacteria.

Finally, when digested sludge was added to a lagoon at Jay, Florida, enteroviruses were readily detected in grab samples from the lagoon. The level of sludge-associated viruses dropped to low or undetectable levels following disposal of sludge on land and during periods when addition of digested sludge to the lagoon was suspended. Enteroviruses were not detected in wells located on the sludge disposal site or near the lagoon.

Overall average quality of sludges used in the project follows:

TABLE 1. CONCENTRATIONS OF METALS IN SEWAGE SLUDGE
PPM-MEANS-DRY MATTER BASIS
1976-1979

	Dried Pensacola Sludge (DPS)	Dried Florida (UF)[a] Sludge (DFS)	Dried Chicago[b] Sludge (DCS)
Cadmium (Cd)	12	13	163
Cobalt (Co)	0.6	7.9	22
Chromium (Cr)	220	218	2,888
Copper (Cu)	548	517	1,365
Iron (Fe)	4,619	9,367	37,267
Lead (Pb)	485	465	774
Mercury (Hg)	7.9	82	5
Nickel (Ni)	35	32	376
Zinc (Zn)	2,440	1,217	2,501

[a] University of Florida

[b] Donated by H. J. Baker and Bros., Inc., Temple Terrace, FL

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RECOMMENDATIONS

Present EPA guidelines on allowable levels of certain contaminants, including metals, would assure availability of homogeneous urban sewage sludges which could be utilized in soil enrichment programs for crop or forestlands. Further research is necessary to assure safe rates and frequency of application of sewage sludges, along with other essential elements, to enhance crop production. If urban sewage sludges for production of certain crops are shown to be contraindicated, this information should be made available. Since certain metals, including cadmium, lead, nickel, and chromium, have been shown to be accumulative in animals consuming forage or grain from sludge-amended soils and therefore have potential hazard to animal health and mankind, it is proposed that further research be done to establish safe guideline levels in feeds intended for meat producing animals.

The presence of *Sarcocystis* sp. in muscle from cattle and swine consuming sludge or forage and grain fertilized with sewage sludge incorporated into their diets suggest that this potential animal and human health hazard may be associated with consumption of urban sludges. Methods to eliminate this hazard or prevent its infectivity must be established prior to utilization of sludges for crop or animal production. Other parasites, including infective stages of ascarids, may persist in sludges. Destruction of parasites or preventive programs to eliminate them from sludge must also be developed. Therefore, it is recommended that research to establish the incidence, diagnosis and factors predisposing to sarcocystis infection in cattle and swine associated with sludge utilization be initiated.

Finally, since viruses hazardous to animal production and human health have been shown to be present in certain urban sewage sludges, further research to characterize these viruses and assure their reduction to nonhazardous levels should be continued and completed expeditiously to allow land application of sewage sludges as plant nutrients.

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EFFECTS OF LIQUID DIGESTED SLUDGE ON SOILS AND PLANTS

SECTION A. The Nutrient Status of Six Field Crops Grown on Soils Treated with Liquid Digested Sludge. M.C. Lutrick

SECTION B. The Utilization of Liquid Digested Sludge on Agricultural Land. M.C. Lutrick

SECTION C. The Uptake of Certain Metals by Corn Grown on Soil Treated with Chicago or Pensacola Sewage Sludge. M.C. Lutrick

SECTION D. Sludge Effects on Yield and Cadmium Uptake of Coastcross 1 Bermudagrass. C.C. Hortenstine

SECTION E. Transport of Sewage Sludge Constituents in a Sandy Soil and Uptake of Metals by Bermudagrass. L.C. Hammond

A. THE NUTRIENT STATUS OF SIX FIELD CROPS GROWN ON SOILS TREATED WITH LIQUID DIGESTED SLUDGE

INTRODUCTION

The disposal of liquid digested sludge (LDS) has become a major problem for many cities. The application of LDS on agricultural land is probably the most practical means of disposal for cities and municipalities. This method eliminates the high cost of alternate means (drying processes) of disposal and an increase in air pollution.

The most extensive studies of LDS used on cropland were reported by Hinesly *et al.* (1971). They concluded that N was the main constituent to consider when using LDS in large quantities. LDS was shown to be an effective source of N, P, and micronutrients. Heavy metals are a ubiquitous constituent of LDS and after application to the soil remain in the plow layer, according to the above authors. Earlier research in Florida by Hortenstine and Rothwell (1973) showed that all of the plant nutrients measured except Mn were increased by compost applications on sorghum.

This study was initiated to measure the effects of liquid Pensacola sludge (LPS), a source of LDS, on the nutrient content of field crops and to establish limits for annual application rates of LDS to crops and soils of west Florida. The results formed a basis for later trials under the EPA project.

METHODS AND MATERIALS

Experiment 1 was a greenhouse study on three Paleudult soils (Red Bay, Dothan, and Troup). Grain sorghum (*Sorghum bicolor* (L.) Moench) was used as the indicator crop. Experiment 2 was also a greenhouse study on a previously limed and well fertilized Red Bay top soil. Corn (*Zea mays* L.), grain sorghum, wheat (*Triticum aestivum* L.), bahiagrass (*Paspalum notatum* Fluegge), peanuts (*Arachis hypogaea* L.), and soybeans (*Glycine max* L.) were grown to determine the nutrient uptake from the LPS.

A field experiment was established on Troup fine sandy loam with 0 and 31.7 metric tons per hectare (t/ha) of LPS applied and incorporated into the soil, on which corn was planted March 23, 1973, and harvested August 30, 1973. The earleaf from the corn of the field experiment was taken when the grain was in the hard dough stage of maturity. Grain samples were taken at harvest. Soil samples were taken at the same time as leaf samples and extracted with double acid so that the micronutrients could be determined as well as P, K, and Mg.

Fresh LPS samples were taken from each delivery and sent to the Analytical Research Laboratory, Soil Science Department, University of Florida, Gainesville, Florida, for analyses.

The analyses of LPS (Table 1) indicate that Zn would be the element most likely to reach levels toxic to plants if large amounts of LPS were added to the soil. The pH range of the LPS was from 6.4 to 7.0. Thus, the LPS would not be expected to have much influence on soil pH except at high rates of application on very acid soils. The importance of LPS as an N source is indicated by the fact that 342 kg/ha of N would be supplied by an application of 6.6 t/ha of LPS.

TABLE 1. ELEMENTAL COMPOSITION OF LIQUID DIGESTED SLUDGE AND AMOUNTS OF ELEMENTS IN A 6.6 T/HA APPLICATION

ELEMENTS	CONTENT* mg/l	 kg/ha
N	1366	342.0
Ca	539	135.0
P	491	123.0
Al	446	112.0
Zn	104	26.0
Fe	77	19.0
Na	67	17.0
Mg	56	14.0
K	50	13.0
Si	46	12.0
Cu	19	4.8
Pb	15	3.8
Cr	8	2.0
Ni	3	0.8
Mn	2	0.5
Ti	2	0.5
Cd	0.55	0.13
Hg	0.55	0.04

* Each value is the average of 5 samples taken at different times

RESULTS AND DISCUSSION

In Experiment 1, the application of up to 80 t/ha of LPS increased the dry matter production of grain sorghum. The most significant effect on forage composition was the reduction of the K concentration in the plants grown on soils to which LPS was applied. This indicates that K probably should be added to the soil to supplement the K added from the application of LPS.

In Experiment 2, corn, grain sorghum, wheat, bahiagrass, peanuts, and soybeans were grown with and without LPS. Dry matter production and uptake of N increased for all crops grown with LPS. The large growth response of soybeans and peanuts suggests the possibility that the LPS must add some element other than N that was deficient in the soil. The elements Na, Fe, Al, and Pb, that could have become toxic when LPS was added to the soil, were generally reduced in the herbage of these crops.

In a field test with corn, the treatments of 0 and 31.7 t/ha of LPS produced 6020 and 7460 kg/ha of grain, respectively. There was very little difference in the protein content of the leaves of the corn grown on the LPS-treated soil compared with the corn grown where no LPS was added. The same was true of the grain.

B. THE UTILIZATION OF LIQUID DIGESTED SLUDGE ON AGRICULTURAL LAND

INTRODUCTION

The application of LPS prior to planting increased the yield of soybeans when compared with commercial fertilizer treatments.

This study was initiated to determine the crop responses, nutrient uptake, and heavy metal accumulation on land treated with LPS.

METHODS AND MATERIALS

Experiments were begun in 1974 using corn (*Zea mays* L.), grain sorghum (*Sorghum bicolor* (L.) Moench), and soybeans (*Glycine max* L.) to determine the effects of liquid Pensacola sludge (LPS) on plant growth and yield. The accumulation of nutrients, certain microorganisms, and heavy metals in plants and soils was determined. The treatments were 0, 20, and 40 t/ha of LPS applied on an annual basis. The 0 LPS treatment received 500 kg/ha of 0-10-20 commercial fertilizer at planting for corn and grain sorghum and 250 kg/ha of ammonium nitrate as a sidedress application. The 0 LPS treatment for soybeans received 500 kg/ha of 0-9-17 commercial fertilizer at planting time. Other LPS treatments received no commerical fertilizer. The experimental design was a randomized complete block with four replications for each crop. Pioneer hybrid '3369A' corn, Funks hybrid 'G-522' grain sorghum, and 'Ransom' soybean were planted on three different Paleudult soils (Troup, Lucy, and Orangeburg, respectively). The soils were sampled to a depth of 150 cm at 30 cm increments. Whole plant samples were taken when the corn, sorghum, and soybeans were at anthesis. Grain samples were taken from all plots at maturity.

Fresh LPS samples were taken periodically, dried, and sent to the Analytical Research Laboratory, Soil Science Department, Gainesville, Florida, for analyses. The composition of the LPS is shown in Table 1. All plant samples were weighed and ashed, and the ash dissolved in 0.1 N HCl. Soil samples were extracted with double acid (0.05 N HCl + 0.02 N H_2SO_4). The P was determined colorimetrically using ammonium molybdate and ascorbic acid as indicated by Watanabe and Olsen (1965). All other determinations were made with either the emission or absorption flame spectrophotometer.

RESULTS AND DISCUSSION

The response of corn and grain sorghum to the amount and method of LPS application was reduced by lack of moisture throughout the growing season. Rainfall was less than normal from March through July, 1974.

The amount of LPS affected the corn and soybean yields but had no significant effect on the yield of grain sorghum.

TABLE 2. THE PRODUCTION OF CORN, GRAIN SORGHUM, AND SOYBEAN ON LPS TREATED SOIL IN 1974

Amount of LPS applied	Corn forage		Sorghum grain	Soybean seed
	Green wt	Dry wt		
t/ha	kg/ha			
0	29,790	9,350	4020	2750
20	29,340	10,100	4090	3370
40	24,190	8,600	3590	2830
	sig quadratic		NS	sig quadratic

Rainfall was adequate for the production of soybeans. Soybeans grown with a commercial fertilizer treatment averaged 15 cm taller than soybeans grown on 20 t/ha LPS treatment but produced less seed (2750 kg/ha, compared with 3370 kg/ha, as shown in Table 2. Half of the 40 t/ha treatment was applied to the plots while the crop was growing. This foliar application coated the small plants and the lack of rainfall allowed the coating to remain on the plants thus reducing the yield of corn, grain sorghum, and soybeans compared with the 20 t/ha treatment, which was all applied prior to planting. The LPS applied at 20 t/ha caused all three crops to mature approximately 1 week earlier than the commercial fertilizer treatment. Concentrations of N, P, and Zn are high in the LPS.

In general, micronutrient and heavy metal composition was higher in plant tissue from LPS-treated plots on all three crops tested. Zinc seemed to be the element most likely to be taken up in the plant and seed in excess since it was found to be very high in the LPS. The analyses of soil treated with LPS indicated that LPS reduced the soil pH and increased P and Zn content.

Analyses of water from Pond Creek and a local spring located near the test site indicated that no detectable amount of nutrients occurred in the runoff waters from the LPS treated land. Water from all wells was low in nutrients, particularly nitrate nitrogen (NO_3-N). The NO_3-N level remained consistently at 0.6 ppm. Water samples taken from the well at the lagoon site (a means of on-farm storage of LPS) indicated that there was some contamination from the LPS in the lagoon.

These preliminary data indicate that LPS can be used on agricultural lands, up to a point, without detrimental effects on crops or cattle consuming forages grown on LPS-treated land. Additional data are needed to determine the amount of LPS required to utilize LPS as a fertilizer.

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C. THE UPTAKE OF CERTAIN METALS BY CORN GROWN ON SOIL TREATED WITH CHICAGO OR PENSACOLA SEWAGE SLUDGE

SUMMARY

Land spreading of sewage sludge is probably the most practical means of disposal for municipalities and cities. However, uptake of certain metals by forage and grain crops from land treated with sludge creates health risks. A 3-year study was conducted to determine the uptake of copper (Cu), zinc (Zn), manganese (Mn), lead (Pb), and cadmium (Cd) by corn leaves and grain from soil treated with Chicago and Pensacola sewage sludge. The dried Chicago sludge (DCS) contained large quantities of Cu, Zn, Pb, and Cd. The Pensacola sludge was high only in Zn.

The quantities of metals extracted from sludge-treated soil were proportional to the quantities added from the sludges. The metal uptake by the corn plant was directly associated with soil pH. The higher the soil pH the smaller the quantity of metal uptake. The quantity of metals extracted from the sludge-treated soil after 1 year was somewhat less than from the soil where sludge had been recently applied. The quantity taken up by the corn leaves was much less from the residual sludge treatment than from the same treatment where sludge had been recently applied.

The concentration of metals in the grain was always much less than the concentration found in the corn leaves from the same sludge treatment. Corn plants from the DCS treatment probably would have contained too much Cd to be utilized for forage. The Pb and Cd concentration in the grain was below detectable limits from all treatments.

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D. SLUDGE EFFECTS ON YIELD AND CADMIUM UPTAKE OF COASTCROSS 1 BERMUDAGRASS

There is widespread interest in the United States in the utilization of municipal sewage sludges as a soil amendment or source of plant nutrients. However, potential hazards exist that must be resolved before sludges can be used to any great extent on agricultural soils. This is particularly true of metals that may enter the human food chain with more or less disastrous effects. Cadmium is especially important in this respect as it can accumulate within various body organs in amounts that can produce diseases or fatalities (Shroeder, 1965; Carroll, 1966; Axelsson and Piscator, 1966). A case in point was the incidence of the "itai itai" disease several years ago in Japan (Tsuchiya, 1969). The occurrence of this disease in a large population of the Jintsu Valley resulted from the daily intake of rice grown on soil which was contaminated by Cd release in effluent from surrounding industries.

The objectives of this study were to:

1. measure Cd movement in and adsorption by a soil treated with sewage sludge of known Cd content,
2. measure uptake of Cd by Coastcross 1 bermudagrass (*Cynodon dactylon* L. Pers.), and
3. compare the sludge to a mineral fertilizer as a source of plant nutrients.

EXPERIMENTAL PROCEDURES

Field plots (5 x 7 m) were established in a 4 x 4 Latin square on Lake sand (Typic Quartzipsamments, hyperthermic, coated) which was sampled prior to sludge or fertilizer applications at 30-cm increments to 360-cm depth. Treatments were:

1. 10-4.4-8.3 (N-P-K) fertilizer applied at 6 t/ha,
2. sewage sludge applied at 12 t/ha,
3. sewage sludge applied at 24 t/ha, and
4. sewage sludge applied at 48 t/ha.

The fertilizer and sludge were disked into the soil and the bermudagrass was sprigged in June, 1977. The fertilizer and sludge were to be reapplied each year after 1977 for a total of 5 years. As sludge is

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notoriously low in K, additional K was applied to the sludge-treated plots at the rate of 560 kg/ha. The bermudagrass was harvested at about 6-week intervals for yield and chemical analyses. Soil was sampled annually at the 0 to 15 and 15 to 30 cm depths for chemical analyses.

Plant tissue samples were dried at 70C, ground in a stainless steel Wiley mill, and ashed for 6 hours at 450C. The ash was dissolved in 1 N HNO_3, transferred to volumetric flasks, and made to volume with distilled deionized water. The solution was analyzed for K, Cu, Mn, Zn, Cd, Ni, and Pb by atomic absorption. Total N was determined by the semimicro-Kjeldahl method (Bremner, 1965) and NO_3-N was determined in a water extract with a specific ion electrode. Phosphorus was determined by the phosphomolybdate stannous chloride method (Jackson, 1958).

RESULTS AND DISCUSSION

The soil used in this study is a deep sand which is generally infertile in the virgin state. As shown in Table 3, the soil was quite low in available plant nutrients with an acid pH to the 360 cm depth. The absence of detectable Cd and extremely low Zn in the extractant used by the University of Florida Soil Science Laboratory indicated that Lake sand is an ideal soil on which to conduct this study as any buildup of these two elements over a period of several years would have to result from fertilizer or sludge applications. Although not shown in Table 3, Pb and Ni were also not detectable in the original soil samples.

The soil in each plot was sampled just prior to the annual application of sludge and fertilizer. Data from DA extracted soil (Table 4) indicate that there was an appreciable increase in most of the elements considered at the end of the second year of the study. Of particular interest was the considerable increase in soil Ca in the 0 to 15 cm depth from sludge applications as compared to the apparent decrease from mineral fertilizer. Sludge applications also increased soil Zn, Cd, Cu, Pb, and Ni with some evidence of movement of these elements below 15 cm depth.

There is presently some controversy in the United States as to the most appropriate extractant for heavy metals in soil. As the available phase of a metal with respect to plant uptake is the determining factor in crop response, we compared the DA method with the method described by Lindsay (1972). Significant (<.001) increases in heavy metal contents of the 0 to 15 cm depth were recorded with both extractants (Tables 4 and 5) and both extractants indicated that some movement of these metals into the 15 to 30 cm depth occurred. Correlations will be calculated between plant uptake and soil content at the end of this study to determine which extractant is better for this type of soil. Heavy metal extraction with 4 M HNO_3 was conducted to give an idea of total metal content (Table 5). The same type of differences was revealed as with the other extractants.

The first full year of growth by the bermudagrass is reported in Table 6. The Cd contents of the bermudagrass in Table 6 are of particular interest. The 30 May harvest was removed before the annual sludge application and Cd contents increased greatly in the second harvest (17 July)

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after the one-half sludge application in May as compared to the first harvest. The second sludge applications were on 1 August and the third harvest on 21 August reflected a still larger increase in Cd uptake.

The yields of each harvest in 1979 and total yields indicate that sludge applied at the 24 ton rate compared favorably with mineral fertilizer as a source of plant nutrients. The highest rate (48 t/ha) of sludge was superior to mineral fertilizer, whereas the 12 ton rate was apparently too low to sustain Coastcross 1 bermudagrass. In fact, the bermudagrass in the 12 ton rate plots died out while bahiagrass became established again.

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TABLE 3. INITIAL CHEMICAL CHARACTERISTICS OF LAKE SAND TO A DEPTH OF 360 CM BEFORE FERTILIZER AND SEWAGE SLUDGE APPLICATIONS

Depth cm	pH*	TSS*	Ca	Mg	P	K	Na	Fe	Zn	Cu	Mn	Mo	Cd
		- - - - - - - - - - - - - - - - - - - µg/g											
0-15	5.1[+]	30	134	21	56	18	40	29	0.95	0.40	5.4	27	0
15-30	4.8	19	78	5	52	8	39	31	0.80	0.40	6.2	17	0
30-60	4.9	20	101	10	34	9	36	32	0.51	0.20	6.4	16	0
60-90	4.8	19	83	7	26	7	36	30	0.63	0.20	6.6	12	0
90-120	4.9	17	104	9	28	10	36	31	0.35	0.20	7.1	18	0
120-150	5.0	17	136	10	33	12	37	31	0.43	0.27	5.5	19	0
150-180	5.0	19	137	14	41	11	34	34	0.40	0.27	4.2	8	0
180-210	5.3	19	168	29	39	15	36	30	0.35	0.27	2.1	8	0
210-240	5.3	25	146	25	33	13	36	30	0.51	0.20	2.5	18	0
240-270	5.2	24	180	76	37	16	39	29	0.60	0.27	1.8	15	0
270-300	4.9	22	214	145	45	18	36	26	0.66	0.20	1.8	7	0
300-330	4.7	25	226	174	45	18	36	28	0.40	0.33	1.9	14	0
330-360	4.5	31	208	158	49	15	35	32	0.51	0.40	1.9	20	0

* pH and total soluble salts were in water (soil + water = 1:2). DA (0.05 N HCl in 0.025 N H_2SO_4) extractant used for all other determinations.

[+] Each entry is an average of three samplings.

TABLE 4. CHEMICAL CHARACTERISTICS OF LAKE SAND (DA EXTRACTED) AT THE END OF 2 YEARS (SAMPLED BEFORE THE THIRD APPLICATION OF FERTILIZER AND SLUDGE).

Sludge applied tons/ha	pH	P	K	Ca	Mg	Fe	Zn	Cd	Cu	Pb	Ni
						0-15 cm μg/g					
0*	5.35+	137	41	69	15	17	3	0	0.2	0	0
12	5.95	81	57	198	41	30	15	0.9	4.0	0.8	0.8
24	5.95	95	66	217	51	44	29	2.0	7.7	1.1	1.6
48	5.83	136	87	402	92	86	84	6.3	18.3	1.5	4.3
s	0.27	27	21	128	30	28	33	2.6	7.1	0.6	1.8
						15-30 cm					
0*	5.30	132	42	37	7	15	1	0	0.2	0	0
12	5.90	68	59	63	15	16	3	0.1	1.0	0	0
24	5.73	75	46	78	20	18	5	0.3	1.5	0.2	0.4
48	5.55	93	34	114	28	29	13	0.8	3.7	0.6	0.9
s	0.24	21	14	39	9	7	6	0.4	1.6	0.3	0.5

* Received 6 tons/ha of 10-4.4-8.3 (N-P-K).

+ Each entry is an average of four samples.

TABLE 5. HEAVY METAL CONTENT OF LAKE SAND (HNO_3 AND DTPA EXTRACTED) AT END OF 2 YEARS (SAMPLED BEFORE THE THIRD APPLICATION OF FERTILIZER AND SLUDGE)

Sludge applied tons/ha	4 M HNO_3							DTPA						
	Fe	Mn	Zn	Cu	Cd	Pb	Ni	Fe	Mn	Zn	Cu	Cd	Pb	Ni
								µg/g 0-15 cm						
0*	660+	32	5	1	0.03	1.8	0.5	49	3.1	1.2	0.3	0	0.1	0
12	1040	39	28	13	1.78	9.9	3.3	46	2.1	8.0	3.1	0.63	0.9	0.3
24	1315	39	42	20	2.89	14.8	5.6	61	3.0	15.9	6.0	1.25	1.6	1.6
48	2865	44	118	58	8.13	36.9	18.9	112	6.6	41.0	15.4	3.25	3.6	5.0
s	930	5	46	23	3.31	15.3	7.9	28	1.8	15.8	6.0	1.28	1.3	2.1
								15-30 cm						
0*	660	32	3	1	0.02	1.7	0.5	28	2.0	0.6	0.3	0	0	0
12	750	33	9	3	0.31	2.0	0.7	28	1.4	1.9	0.9	0.13	0.2	0
24	760	33	11	5	0.51	3.0	1.3	27	1.5	3.0	1.4	0.22	0.2	0
48	960	36	24	11	1.45	6.8	2.9	55	2.8	8.9	3.5	0.64	0.5	0.4
s	150	4	9	4	0.62	2.5	1.1	16	0.7	4.0	1.4	0.30	0.2	-

* Received 6 tons/ha of 10-4.4-8.3 (N-P-K).

+ Each entry is an average of four samples.

TABLE 6. DRY MATTER (70C) YIELD AND SELECTED MINERAL CONTENT OF COASTCROSS 1 BERMUDAGRASS GROWN ON LAKE SAND IN 1978

Sludge applied tons/ha	Yield kg/ha	P	K	Ca	Mg	Cu	Mn	Zn	Cd
		%				µg/g			
					30 May				
0*	Yield date lost	0.11+	1.27	0.26	0.11	3.8	65	20.3	0.35
12		0.11	1.29	0.28	0.13	4.4	60	27.2	1.15
24		0.13	1.53	0.27	0.15	5.0	70	28.1	1.42
48		0.14	1.79	0.29	0.16	5.9	120	33.3	1.81
s		0.02	0.24	0.02	0.02	0.9	8	7.6	0.61
					21 August				
0*	No yield data due to army-worms	0.34	2.75	0.53	0.09	5.5	23	113	0.63
12		0.29	3.95	0.51	0.16	8.8	70	178	3.63
24		0.30	3.43	0.41	0.16	9.0	70	115	6.05
48		0.34	3.35	0.48	0.15	9.5	87	151	6.47
s		0.03	0.39	0.15	0.03	1.8	26	67	2.72
					12 December				
0*	2685	0.27	2.39	0.31	0.14	5.0	279	51	0.14
12	1750	0.22	1.65	0.31	0.14	6.8	121	65	0.96
24	2950	0.27	2.44	0.33	0.20	8.4	193	86	2.33
48	4040	0.30	2.87	0.34	0.26	9.5	215	103	4.30
s	1010	0.04	0.47	0.02	0.05	1.8	63	25	1.68

* Received 6 tons/ha of 10-4.4-8.3 (N-P-K).

+ Each entry is an average of four samples.

E. TRANSPORT OF SEWAGE SLUDGE CONSTITUENTS IN A SANDY SOIL AND UPTAKE OF METALS BY BERMUDAGRASS

Commercial Chicago sewage sludge (dried) was applied to bermudagrass growing on a well-drained sandy soil (coated, hyperthermic Typic Quartzipsamment) at Gainesville, Florida. Annual rates for 3 years on 4 respective treatments were 0, 12, 24, and 48 tons/ha. The no-sludge treatment received 6 tons/ha annually of a chemical fertilizer (10-4.4-8.3, N-P-K). Forage was harvested for yield and chemical analysis at about 6-week intervals during the growing season. Frost-killed sod remained undisturbed during the winter. Rainfall, irrigation, and estimated evapotranspiration data were used in a computer model to estimate the leaching potential for the soil-plant-climate system over the 3-year period.

Forage yields increased each year and in 1979 they were 20, 14, 20, and 26 thousand kg/ha for the 0, 12, 24, and 48 tons/ha sludge rates. For these respective treatments in 1979, uptake of Cd was 3, 22, 46, and 101 mg/ha and uptake of Zn was 413, 501, 788, and 1530 mg/ha.

Metals have accumulated in the 0 to 15 cm soil profile; Cd = 6.3 μg/g and Zn = 83 μg/g at the highest sludge rate. Transport into the 15 to 30 cm zone has occurred; Cd = 0.8 μg/g and Zn = 12 μg/g. These results are in line with the low leaching potential calculated from the rainfall amounts and distribution over the 3 year period. The following tables (7-9) illustrate these results.

TABLE 7. OVEN-DRY YIELDS OF COASTCROSS 1 BERMUDAGRASS (*Cynodon dactylon* L. Pers.) ON LAKE SAND (TYPIC QUARTZIPSAMMENTS, HYPERTHERMIC, COATED)

	Material applied			
Year	10-10-10 (6)*	Sludge (12)*	Sludge (24)*	Sludge (48)*
	kg/ha			
1977				
1st cut	2451	1017	649	0
2nd cut	3800	4046	3760	2769
3rd cut	1260	1240	1334	2671
TOTALS	7511	6303	5743	5440
1978				
1st cut	6260	5343	5963	5797
2nd cut	6049	4715	6760	7049
3rd cut	2683	1746	2949	4037
TOTALS	15092	11804	15672	16883

* Metric tons/ha.

TABLE 8. MINERAL CONSTITUENTS IN COASTCROSS 1 BERMUDAGRASS ON LAKE SAND

Year	K	Ca	Mg	Cu	Mn	Zn	Cd
	%				ppm		
1977							
1st cut:							
10-10-10	6.33	0.46	0.29	9	231	80	0.35
Sludge-12	6.57	0.53	0.38	18	275	116	1.67
Sludge-24	5.99	0.51	0.39	31	313	114	2.45
Sludge-48	--	--	--	--	--	--	--
2nd cut:							
10-10-10	4.66	0.56	0.30	8	202	16	0.33
Sludge-12	5.35	0.54	0.34	9	221	23	0.35
Sludge-24	5.41	0.54	0.32	10	247	26	0.69
Sludge-48	6.00	0.51	0.34	11	465	32	1.58
3rd cut:							
10-10-10	5.70	0.85	0.45	15	277	29	0.35
Sludge-12	5.34	0.80	0.43	17	251	46	0.35
Sludge-24	5.70	0.78	0.43	18	288	49	0.44
Sludge-48	6.10	0.62	0.41	19	395	65	2.20
1978							
1st cut (5/30):							
10-10-10	1.27	0.26	0.11	3.8	65	20	0.35
Sludge-12	1.29	0.28	0.03	4.4	60	27	1.15
Sludge-24	1.53	0.27	0.15	5.0	70	28	1.42
Sludge-48	1.79	0.29	0.16	5.9	120	33	1.81
2nd cut (7/17):							
10-10-10	2.27	0.15	0.11	5.4	103	104	0
Sludge-12	2.07	0.16	0.12	6.2	74	225	2.60
Sludge-24	2.46	0.16	0.13	7.5	89	174	3.30
Sludge-48	2.85	0.18	0.16	9.5	133	149	3.50
3rd cut (8/21):							
10-10-10	2.75	0.53	0.09	5.5	123	162	0.62
Sludge-12	2.95	0.51	0.16	8.8	70	178	3.62
Sludge-24	3.43	0.41	0.16	9.0	70	115	6.05
Sludge-48	3.35	0.48	0.15	9.5	87	151	6.47
4th cut (10/25):							
10-10-10	1.76	0.33	0.14	6.7	218	99	--
Sludge-12	1.39	0.23	0.13	7.4	85	73	--
Sludge-24	1.97	0.28	0.16	8.5	268	90	--
Sludge-48	2.16	0.29	0.18	8.5	172	87	--
5th cut (12/12):							
10-10-10	2.39	0.31	0.14	5.0	279	52	--
Sludge-12	1.65	0.31	0.14	6.8	121	65	--
Sludge-24	2.44	0.33	0.20	8.4	193	100	--
Sludge-48	2.87	0.34	0.26	9.5	215	103	--

TABLE 9. SELECTED SOIL CONSTITUENTS IN LAKE SAND AT END OF FIRST YEAR (1977)

Treatment	pH	Ca	Mg	K	Na	NO_3-N	Cu	Zn	Cd
	- - -	- - -	- - -	- - -	ppm	- - -	- - -	- - -	- - -
0-15 cm									
10-10-10	5.6	103	22	41	10	5.4	0.7	2.8	0.1
Sludge-12	5.8	160	40	43	13	6.0	4.1	10.5	0.9
Sludge-24	6.0	201	49	44	14	5.4	6.3	16.3	1.0
Sludge-48	6.0	243	77	55	18	6.6	11.2	29.5	2.0
15-30 cm									
10-10-10	5.3	25	6	26	9	4.4	0.5	1.8	0.1
Sludge-12	5.4	25	6	34	13	4.2	0.6	1.9	0.1
Sludge-24	5.4	26	8	28	11	4.1	0.6	1.7	0.1
Sludge-48	5.2	27	11	21	9	4.8	1.2	2.2	0.1

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CATTLE FEEDING TRIALS WITH SLUDGES OR FEED GROWN ON SLUDGE TREATED LAND

SECTION A. Dried Pensacola Liquid Digested Sludge in the Diet of Steers. J. Bertrand, M. C. Lutrick, and G. T. Edds, 1974

SECTION B. Winter Annual Pastures Fertilized with Pensacola Liquid Digested Sludge and Grazed by Growing Beef Steers. J. Bertrand, M. C. Lutrick, and H. T. Nguyen, 1976

SECTION C. Dried Pensacola Liquid Digested Sludge (DPS) in the Diets of Feedlot Steers. J. Bertrand, H. T. Nguyen, and H. Breland

SECTION D. Dried Chicago Sludge and Corn from Soil Fertilized with Liquid Pensacola Sludge in Diets of Beef Steers. J. Bertrand, M. C. Lutrick, H. T. Nguyen, and H. Breland, 1977

SECTION E. Forage Sorghum Silages Grown on Soil Treated with Liquid Digested Sludge and Fed to Beef Steers. J. Bertrand, M. C. Lutrick, O. Osuna, G. T. Edds, S. West, and J. Devore, 1978

SECTION F. Pensacola Bahiagrass Pastures Fertilized with Pensacola Liquid Digested Sludge and Grazed by Beef Steers. J. Bertrand, M. C. Lutrick, O. Osuna, and J. Devore, 1979

The initial research at Jay, Florida on the comparative effectiveness of Pensacola liquid digested sludge (LPS) and commerical fertilizers for soil improvement indicated equal effectiveness. However, it was not known whether certain agents or elements in the sludge would be translocated into the plant forage or grain with potential hazard(s) to animals consuming this feed. Therefore, a series of trials were run by Bertrand, *et al.*, in beef steers under feedlot or grazing operations to determine whether growth performance or carcass quality would be adversely affected.

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A. DRIED PENSACOLA LIQUID DIGESTED SLUDGE IN THE DIET OF STEERS

ABSTRACT

Six steers were randomly allotted and fed one of two diets for 219 days to determine the effects of dried Pensacola liquid digested sludge (DPS) on animal performance and concentrations of selected elements in liver, muscle, and kidney tissues. The experimental diets consisted of the control diet and the treated diet (100 grams per head daily of DPS incorporated into the control diet prior to feeding). Steers fed the control diet gained slightly faster and were slightly more efficient in converting feed to gain than those fed the treated diet. Feed consumption was higher for steers on the control diet. No significant differences in performance between the diets were detected.

The concentrations of a number of the elements, aluminum (Al), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), nickel (Ni), and zinc (Zn), were lower in the livers of steers receiving DPS in their diet. The Al, cadmium (Cd), and lead (Pb) concentrations were higher in the kidneys of steers fed DPS.

EXPERIMENTAL PROCEDURES

Six steers, average 192 kilograms (kg), of Angus and Holstein breeding were weighed and allotted at random to two experimental groups of 3 steers each. The control group received the control diet listed in Table 1; while the treated group received the control diet plus 100 grams (g) per head daily of DPS incorporated into the diet prior to feeding. The DPS was sun-dried to approximately 6% moisture in shallow vats located in greenhouses.

After an overnight shrink, individual animal weights were obtained at the beginning and end of the experiment. Group weights were obtained every 28 days during the period.

The steers were fed *ad libitum* once daily for a total of 219 days (April 19 to November 24, 1974).

At the end of the experimental period, the steers were slaughtered at the University of Florida Meats Laboratory, Gainesville. Liver, muscle, and kidney tissues were collected at slaughter.

The DPS and tissue samples (liver, muscle, and kidney) were sent to the Analytical Research Laboratory, Gainesville, for elemental analyses. Concentrations of selected elements in the DPS are listed in Table 2.

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Analyses of variance for animal performance data and concentrations of selected elements in tissues were conducted according to the method of Snedecor (1946).

RESULTS AND DISCUSSION

Performance data with steers fed the two diets for a 219-day feeding period are shown in Table 3. Steers fed the control diet gained slightly faster than steers fed the treated diet (1.03 and 0.93 kg/head daily, respectively). The differences were not significant. Steers fed the control diet were also slightly more efficient in converting feed to gain than those fed the treated diet (8.5 versus 8.8 units of feed per unit, respectively). Feed consumption was higher for the steers on the control diet.

The concentrations of a number of the elements Al, Cu, Fe, Mg, Mn, Ni, and Zn were lower in the livers of steers receiving DPS in their diet (Table 4). These reduced elemental concentrations in livers of steers receiving DPS in their diet were all statistically significant except for Fe and Ni. There were no differences in the concentrations of the selected elements in muscle tissues between the two diets. The Al, Cd, Pb concentrations were higher ($P < 0.01$) in the kidney tissues of steers fed DPS and were a matter of concern.

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(PREVIOUSLY CIN. EPA FORM 287)
TYPING GUIDE SHEET

TABLE 1. CONTROL DIET CONSUMPTION[a]

Ingredients	International Ref. No.	Percent
Sorghum, Milo (*Sorghum vulgare*) grain, pound	4-04-444	68.03
Urea - 45% N		0.45
Soybeans (*Glycine max*) seeds, solvent-extracted, ground, 44%	5-05-604	4.50
Trace mineral salt[b]		0.45
Phosphate - defluorinated, ground	6-01-780	1.34
Oats (*Avena sativa*) - hay, s-c	1-03-280	25.23
Antibiotic supplement[c]		+
Vitamin A palmitate[d]	7-05-143	+

[a] As-fed basis.

[b] Contained not less than 0.350% Zn, 0.340% Fe, 0.200% Mn, 0.033% Cu, 0.007% I, and 0.005% Co.

[c] Zinc bacitracin added at the level of 19.8 mg/kg of diet.

[d] Supplied 3308 IU/kg of diet.

TABLE 2. CONCENTRATIONS OF SELECTED ELEMENTS IN DRIED PENSACOLA LIQUID DIGESTED SLUDGE[a]

Elements	μg/g[b]
Al	13,500
Ca	16,300
Cd	20
Co	10
Cr	240
Cu	570
Fe	2,330
Hg	5
K	1,510
Mg	1,690
Mn	50
N	41,400
Na	2,030
Ni	80
P	14,900
Pb	460
Si	1,400
Ti	50
Zn	3,150

[a] Solids averaged 3.3%.

[b] Dry matter basis.

TABLE 3. PERFORMANCE DATA WITH STEERS RECEIVING THE FEEDLOT DIETS

Item	Control diet	Treated diet[a]
Number of animals	3	3
Length of trial, days	219	219
Average initial weight, kg	190	194
Average final weight, kg	416	398
Average gain/animal, kg	226	204
Average daily gain, kg	1.03	0.93
Feed/gain ratio	8.5	8.8
Feed/animal/day, kg	8.8	8.3

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[a] DPS was added at the level of 100 g/animal/day to the control diet.

TABLE 4. CONCENTRATIONS OF SELECTED ELEMENTS IN LIVER, MUSCLE, AND KIDNEY TISSUES[a]

Elements	Liver Control	Liver Treated	Muscle Control	Muscle Treated	Kidney Control	Kidney Treated
	- - - - - - - - -		μg/g (dry matter basis)		- - - - - - - - -	
Al	36.06^c	16.74^b	57.42	70.25	38.37^d	57.27^e
As	0	0	0	0	0	0
Ba	0	0	0	0	0	0
Ca	72.94	80.68	63.17	65.21	219.71	242.55
Cd	0.27	0.28	0.03	0.04	1.39^d	1.93^e
Co	0	0	0	0	0	0
Cr	0.06	0.06	0.54	0.67	0.43	0.55
Cs	7.39	7.37	8.16	8.46	8.60	9.44
Cu	91.98^e	15.74^d	4.08	3.57	19.41	19.27
Fe	43.03	27.31	85.52	83.19	243.54	261.99
Mg	455.81^e	192.24^d	773.81	799.18	721.69	782.61
Mn	6.96^c	4.66^b	0.85	1.05	5.53	5.82
Mo	2.81	2.29	0	0	1.60	1.79
Ni	0.46	0.15	0.09	0.33	0.31	0.10
Pb	1.79	1.83	1.08	1.27	2.59^d	6.90^e
Se	0	0	0	0	0	0
Si	1.49	1.14	0.55	0.62	0.95	1.10
Sr	0	0	0	0	0	0
Ti	0	0	0	0	0	0
Zn	111.98^e	54.07^d	203.71	179.11	84.11	86.88

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[a] Data presented as means of three observations.

[b,c] Means in a row for a particular tissue with different superscripts differ significantly ($P < 0.05$).

[d,e] Means in a row for a particular tissue with different superscripts differ significantly ($P < 0.01$).

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B. WINTER ANNUAL PASTURES FERTILIZED WITH PENSACOLA LIQUID DIGESTED SLUDGE AND GRAZED BY GROWING BEEF STEERS

Winter annual pastures (a mixture of wheat and crimson clover) rotationally grazed by growing beef steers were fertilized with normal amounts of fertilizer (control) or Pensacola liquid digested sludge (LPS) applied by two methods: 1. LPS #1 - 19.8 t/ha of LPS incorporated into the soil prior to the planting, and 2. LPS #2 - 6.6 t/ha of LPS incorporated into the soil prior to planting and 13.2 t/ha of LPS topdressed on the soil and forage during the grazing season. The steers grazing the pastures receiving the LPS #1, Control, and LPS #2 treatments had average daily gains of 0.97, 0.88, and 0.84 kg per head, respectively. The gains per hectare were 487, 417, and 359 kg for the steers. Incorporation of LPS into the soil prior to planting was beneficial to forage growth while topdressing with LPS on the soil and forage was detrimental due to trampling by the tractor and sludge spreader, decreased growth by LPS-coated leaves, and the initial killing of much of the young crimson clover.

The wheat was planted with a grain drill at the rate of 140 kg/ha. The crimson clover was seeded over the wheat with a cultipacker-seeder at the rate of 9 kg/ka. The 1.0 ha plots were planted on October 23 and 24, 1975; while the 0.5 ha plots were planted on November 5, 1975.

The trial was initiated on December 19, 1975, and terminated when the forage was essentially grazed out. Due to a long and consistent cold period and slow forage growth, each group of steers had to be removed from the experimental plots for a short period during the early part of 1976 to allow the forage to recover.

Individual animal weights were taken at the beginning and end of the trial period after an overnight shrink. Group weights were obtained every 28 days at approximately the same time of day. Additional grazer animals were added and removed as needed to keep the forage uniformly grazed. Each experimental group of steers was rotated between the two pasture plots assigned to it as required for best utilization of good quality forage.

A mineral mixture (consisting of 2 parts defluorinated rock phosphate and 1 part trace-mineralized salt), plain salt, and clean drinking water were available to the animals at all times.

One group of 4 steers from each treatment was slaughtered on April 21, 1976; while 1 group of 4 steers from each treatment was slaughtered at the University of Florida Meats Laboratory, Gainesville, on May 15, 1976.

An analysis of variance on the average daily gain data was conducted according to the method of Snedecor (1946).

RESULTS AND DISCUSSION

The performance data of growing beef steers grazing winter annual pastures fertilized with LPS are presented in Table 5. Steers grazing the pastures receiving the LPS #1 treatment had an average daily gain of 0.97 kg per head for a period of 128 days, followed by an average daily gain of 0.88 kg per head for steers grazing the control pastures for a period of 121 days, and an average daily gain of 0.84 kg per head for steers grazing the pastures receiving the LPS #2 treatment for a period of 121 days. There were no significant differences in the average daily gain between treatments.

The larger gain per hectare for steers grazing the pastures receiving the LPS #1 treatment was due to a longer grazing period. Steers grazing the pastures receiving the LPS #2 treatment had a low gain per hectare, mainly because of a low daily gain and a low stocking rate per hectare. The top-dressing with 13.2 t/ha of LPS during the grazing period appeared to be detrimental to the forage on these pastures from the standpoint of trampling by the tractor and sludge spreader and poor forage growth. Coating the surfaces of the leaves with LPS appeared to decrease forage growth; this was apparently due to decreased photosynthesis. Thus, the use of LPS as a fertilizer on agricultural land intended for use as winter annual pastures for grazing by growing beef steers was beneficial if the LPS was incorporated into the soil prior to planting.

TABLE 5. PERFORMANCE DATA OF GROWING BEEF STEERS GRAZING WINTER ANNUAL PASTURES FERTILIZED WITH PENSACOLA LIQUID DIGESTED SLUDGE

	Treatments		
Item	Control[a]	LPS #1[b]	LPS #2[c]
Initial number of animals	8[d]	8	8
Length of grazing, days	121[e]	128[f]	121[e]
Average initial weight, kg	202	190	198
Average final weight, kg	309	314	300
Average gain/animal, kg	107	124	102
Average daily gain, kg	0.88	0.97	0.84
Animal days/ha[g]	474	502	427
Stocking rate/ha[g]	3.9	3.9	3.5
Gain/ha, kg[h]	417	487	359
Gain/ha/day, kg[h]	3.43	3.78	2.94

[a] Rotational grazing of a wheat and crimson clover mixture - 560 kg/ha of 14-12-12 fertilizer at planting time and 3 applications of 112 kg/ha each of ammonium nitrate during the grazing season.

[b] Same pasture mixture as above - fertilized with 19.8 t/ha of LPS prior to planting.

[c] Same pasture mixture as above - fertilized with 6.6 t/ha of LPS prior to planting and topdressed with 13.2 t/ha of LPS during the grazing season.

[d] Initially, 2 groups of 4 steer calves each.

[e] One group of calves grazed for 109 days; while the other group grazed for 133 days.

[f] One group of calves grazed for 116 days; while the other group grazed for 140 days.

[g] Additional grazer animals of the same type and size were added and removed as needed to keep the forage uniformly grazed.

[h] Grazer steer gain was considered at the same rate as that of the experimental animal gain.

C. DRIED PENSACOLA LIQUID DIGESTED SLUDGE (DPS) IN THE DIETS OF FEEDLOT STEERS

ABSTRACT

Twenty-four beef steers of British breeding were randomly allotted and fed 1 of 3 diets for 119 days to determine the effects of dried Pensacola liquid digested sludge (DPS) incorporated into the diets at feeding time on animal performance, carcass quality, and concentrations of selected, potentially toxic metals in liver, muscle, and kidney tissues. The 3 levels of dried DPS were 0, 250, and 500 grams (g) per head daily. The performance and carcass data of steers fed the 250 and 500 g per head daily were not significantly different from those of the control steers. The cobalt (Co), copper (Cu), nickel (Ni), and zinc (Zn) concentrations in liver tissues were significantly lower, while the lead (Pb) concentrations were significantly higher, for steers receiving the high level of DPS. There were some accumulations of Pb in kidney tissues of DPS-treated animals.

EXPERIMENTAL PROCEDURES

Twenty-four beef steers, average 290 kilograms (kg), of British breeding (Angus and Hereford) were allotted at random from breed groups to 6 experimental groups of 4 steers each. The 6 experimental groups, 2 groups (replicates) per treatment, were assigned to 3 DPS diet levels of 0, 250, and 500 g per head daily.

The control diet consisted of 73.5% corn (*Zea mays*) - aerial part, ensiled, mature, well-eared mx 50% mn 30% dry matter, International reference no. 3-08-153 (41.5% dry matter), 23.9% ground corn, dent yellow (*Zea mays indentata*) - grain, gr 2 US, International reference no. 4-02-931 (88.4% dry matter), and 3.1% concentrate supplement mixture (87.7% dry matter) on an as-fed basis. The diet was 56% corn silage, 39% ground corn, and 5% concentrate supplement mixture on a dry matter basis. The ingredients of the concentrate supplement mixture are listed in Table 6.

Individual animal weights were obtained at the beginning and end of the trial after an overnight shrink. Group weights were obtained every 28 days during the period. These weights were obtained in order to periodically check on the performance of the steers. Blood, fecal, feed ingredient, and DPS samples were collected each time for analysis.

The trial was initiated on August 19 and terminated on December 16, 1976 (119 days). The steers were fed *ad libitum* twice daily. The DPS was incorporated into the diets at each feeding. The amounts of feed for each group of steers were recorded at each feeding.

The DPS was sun-dried to approximately 6% moisture in shallow vats located in greenhouses.

The steers were slaughtered at the University of Florida Meats Laboratory, Gainesville. Liver, muscle, and kidney tissues were collected at slaughter for metal analysis. Carcass measurements were secured.

The DPS, feed ingredients, blood, fecal, and tissue (liver, muscle, and kidney) samples were sent to the Analytical Research Laboratory, Soil Science Department, University of Florida, Gainesville, for metal analyses and the blood was also evaluated as to hematology and clinical chemistry. Concentrations of selected metals in DPS and feed ingredients in the diets are listed in Table 7.

Analyses of variance for animal performance and carcass quality data were conducted according to the method of Snedecor (1946). The computer package (Statistical Analysis System, North Carolina State University) outlined by Barr *et al.* (1976) was utilized for the statistical analyses of the metal data in blood, feces, and tissues.

RESULTS AND DISCUSSION

Performance and carcass data with beef steers fed the 3 DPS levels (0, 250, and 500 g/head/day) in their feedlot diets for a 119-day period are listed in Table 8. Steers fed the control diet had an average daily gain of 0.80 kg/head, followed by 0.77 kg/head for steers receiving 250 g/head daily, and 0.72 kg/head for steers receiving the 500 g/head daily. There were no differences among treatments in average daily gain.

Steers receiving the 250 and 500 g/head daily in their diets were 2.4 and 6.7% less efficient in converting feed to gain than the control steers (Table 8). The high level of DPS slightly depressed feed intake.

On a dry matter basis, the diets containing 250 and 500 g/head daily contained 3.3 and 6.8% of DPS. Studies at Maryland indicated that as much as 5 to 6% of the diet, on a dry matter basis, of animals grazing liquid sludge-treated pastures could consist of sludge adhering to edible plant surfaces (A. M. Decker, personal communication). This amount would depend upon the prevalence of rain following each application.

There were no differences among treatments as far as any of the carcass parameters were concerned.

Concentrations of selected metals in feces are shown in Table 9. There were no differences among treatments. However, significant differences existed for metals in fecal material. Metal concentrations increased as the level of DPS incorporated into the diets increased. Excretion in the feces played an important part in the elimination of excessive amounts of metals in the diets.

Concentrations of selected metals in liver and kidney tissues are shown in Table 10. The cobalt (Co), copper (Cu), nickel (Ni), and zinc (Zn) concentrations in liver tissues were significantly lower for steers receiving the high level of DPS. The high level of DPS may have had a detrimental effect on the liver storage mechanism for these metals. However, the lead (Pb) concentrations were significantly higher in the livers of steers receiving the high level of DPS. Cobalt was the only metal significantly higher in muscles of steers receiving DPS than in control animals. Lead levels increased in kidney tissues of DPS-treated animals.

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TABLE 6. CONCENTRATE SUPPLEMENT MIXTURE[a]

Ingredients	International Reference Number	Percent
Soybean (*Glycine max*) - seeds, solvent extracted, ground, 44%	5-04-604	60.5
Urea - 45% N		12.6
Phosphate - defluorinated, ground	6-01-780	17.9
Trace mineral salt[b]		9.0
Vitamin A palmitate[c]	7-05-143	+

[a] As-fed basis.

[b] Contained not less than 0.350% Zn, 0.340% Fe, 0.200% Mn, 0.033% Cu, 0.007% I, and 0.005% Co.

[c] Supplied 64,500 IU/kg of concentrate supplement mixture.

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PAGE NUMBER
EPA Form 2550-4
TYPING GUIDE SHEET

TABLE 7. CONCENTRATIONS OF SELECTED METALS IN DPS AND THE FEED INGREDIENTS IN THE FEEDLOT DIETS

Metals	Pensacola sludge[a]	Ground corn	Corn silage	Concentrate supplement mixture
	- - - - - - - - - -	- - - - - - - - µg/g[b]	- - - - - - - -	- - - - - - - - - -
Cd	7	<.025	<.025	2.88
Co	10	< .10	< .10	12.00
Cr	192	< .10	< .10	15.90
Cu	309	1.25	3.00	25.52
Hg	5	--[c]	--	--
Ni	20	< .10	< .10	10.50
Pb	193	< .20	< .20	4.00
Zn	2570	21.5	18.4	283.8

[a] Solids averaged 3.3%.

[b] Dry matter basis.

[c] Not analyzed.

TABLE 8. PERFORMANCE AND CARCASS DATA WITH BEEF STEERS RECEIVING VARIOUS LEVELS OF DPS IN FEEDLOT DIETS[a]

Item	Treatments (g/head/day)[b]		
	0	250	500
Number of animals	8[c]	8	8
Length of trial, days	119	119	119
Average initial weight, kg	288	292	290
Average final weight, kg	383	384	376
Average daily gain, kg	0.80 ± 0.16	0.77 ± 0.07	0.72 ± 0.21
Feed/gain ratio	16.5	16.9	17.6
Feed/animal/day, kg	13.2	13.2	12.7
Average carcass quality grade[d]	15.5 ± 3.3	14.5 ± 2.3	15.4 ± 2.7
Average carcass yield grade	2.8 ± 0.4	2.7 ± 0.5	2.7 ± 0.4

[a] Data presented as means ± SD where appropriate for 8 observations.

[b] Grams per head daily of dried Pensacola liquid digested sludge incorporated into the diet.

[c] Two groups of 4 steers each.

[d] 14 = average good, 15 = high good, 16 = low choice.

TABLE 9. METALS IN FECES[a]

	Treatments (g/head/day)[b]		
	0	250	500
Number of observations	24	19	23
Metals in feces	- - - - - - - -	µg/g (dry matter basis)	- - - - - - - -
Cd	$0.72^{f} \pm 0.28$	$1.93^{g} \pm 0.77$	$2.43^{h} \pm 0.66$
Co	$2.53^{c} \pm 0.65$	$3.71^{d} \pm 1.18$	$3.75^{d} \pm 1.18$
Cr	$5.54^{f} \pm 2.05$	$30.89^{g} \pm 8.83$	$43.73^{h} \pm 13.44$
Cu	$12.97^{f} \pm 3.02$	$58.91^{g} \pm 15.24$	$90.00^{h} \pm 20.19$
Hg	$.01^{f}$	$0.68^{g} \pm 0.48$	$1.48^{h} \pm 0.63$
Ni	3.77 ± 1.65	7.95 ± 4.51	9.11 ± 3.93
Pb	$2.75^{c} \pm 3.36$	$45.57^{d} \pm 19.23$	$63.27^{e} \pm 43.63$
Zn	$96.8^{f} \pm 29.9$	$250.7^{g} \pm 54.1$	$345.5^{h} \pm 87.2$

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[a] Data presented as means ± SD where appropriate.

[b] Grams per head daily of dried Pensacola liquid digested sludge incorporated into the diet.

[c,d,e] Means in a row with different superscripts differ significantly ($P < 0.05$).

[f,g,h] Means in a row with different superscripts differ significantly ($P < 0.01$).

EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 287)
TYPING GUIDE SHEET

TABLE 10. CONCENTRATIONS OF SELECTED METALS IN LIVER AND KIDNEY TISSUES[a]

	Treatments (g/head/day)[b]		
	0	250	500
Number of observations	8	8	8
Metals in liver tissues	- - - - - - - - - - - -	μg/g[c]	- - - - - - - - - - - -
Cd	0.14 ± 0.04	0.20 ± 0.06	0.16 ± 0.04
Co	0.35^{g} ± 0.01	0.36^{g} ± 0.03	0.14^{f} ± 0.02
Cr	< .10	< .10	< .10
Cu	$12.67^{d,e}$ ± 9.06	17.07^{e} ± 5.88	8.34^{d} ± 4.24
Hg	< .01	< .01	< .01
Ni	0.32^{g} ± 0.09	0.29^{g} ± 0.11	0.14^{f} ± 0.02
Pb	0.27^{f} ± 0.19	0.26^{f} ± 0.16	0.58^{g} ± 0.07
Zn	44.01^{g} ± 11.10	44.91^{g} ± 7.74	18.00^{f} ± 11.90
Metals in kidney tissues			
Cd	0.27 ± 0.10	0.38 ± 0.08	0.40 ± 0.09
Co	0.24 ± 0.13	0.13 ± 0.09	0.20 ± 0.10
Cr	< .10	< .10	< .10
Cu	3.83 ± 0.60	4.03 ± 0.26	3.79 ± 0.48
Hg	< .01	< .01	< .01
Ni	< .10	≤ .13 ± 0.08	≤ .16 ± 0.11
Pb	1.40^{d} ± 0.14	1.95^{e} ± 0.49	$1.66^{d,e}$ ± 0.32
Zn	18.79 ± 2.22	19.66 ± 0.97	19.09 ± 2.16

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[a] Data presented as means ± SD where appropriate. [b] Grams per head daily of DPS incorporated into the diet. [c] Fresh tissue basis. [d,e] Means in a row with different superscripts differ significantly ($P < 0.05$). [f,g] Means in a row with different superscripts differ significantly ($P < 0.01$).

D. DRIED CHICAGO SLUDGE AND CORN FROM SOIL FERTILIZED WITH LIQUID PENSACOLA SLUDGE IN DIETS OF BEEF STEERS

ABSTRACT

Twenty-four steers were randomly allotted and fed 1 of 3 diets for 141 days to determine the effects of digested municipal sludges on animal performance, carcass quality, and concentrations of selected metals in liver, muscle, and kidney tissues. The experimental diets consisted of (1) control corn diet, (2) "spiked" corn diet - control corn diet plus 500 grams (g) per head daily of dried Chicago digested sludge (DCS) high in cadmium (Cd), and (3) LPS corn diet - corn produced from soil fertilized with surface applications totaling 19.8 metric tons/hectare (t/ha) of Pensacola liquid digested sludge (LPS) prior to planting.

Inclusion of DCS in the diet or corn grown from soil fertilized with LPS had no effect on growth performance nor carcass quality measurements with beef steers. No significant differences in concentrations of selected metals were detected in livers and kidneys of steers fed the control corn diet and the LPS corn diet. The Cd, copper (Cu), iron (Fe), and lead (Pb) concentrations in livers were higher ($P < 0.01$ for Cd, Cu, and Fe; $P < 0.05$ for Pb) with steers fed the "spiked" corn diet. Accumulations ($P < 0.01$) of Cd, Fe, mercury (Hg), and Pb occurred in kidneys of steers fed the "spiked" corn diet compared with the other 2 experimental diets.

Higher ($P < 0.01$) Cd concentrations in livers and kidneys of steers were observed from feeding the diet containing DCS. Since Cd exposure can cause kidney damage, the Cd content of a sewage sludge could determine the amount that may be safely applied to agricultural land.

INTRODUCTION

The purpose of this study was to determine the effects of dried Chicago digested sludge (DCS), high in Cd, and corn produced on soil fertilized with Pensacola liquid digested sludge (LPS) in feedlot diets of beef steers on animal performance, carcass quality, and concentrations of selected metals in liver, muscle, and kidney tissues.

EXPERIMENTAL PROCEDURES

Twenty-four beef steers, average 299 kilograms (kg), of British breeding (Angus and Angus X Hereford crosses) were allotted at random from breed groups to 6 experimental groups of 4 steers each. The 6 experimental groups, utilizing 2 groups (replicates) per treatment, were assigned to 3 corn diets: (1) control corn, dent yellow (*Zea mays indentata*)

- grain, gr 2 US, International Reference No. 4-02-931; (2) control corn plus 500 g per head daily of DCS incorporated into the diet prior to feeding; and (3) corn produced from soil fertilized with surface applications of LPS totaling the equivalent of 19.8 t/ha of dry material prior to planting. The sun-dried sludge was plowed into the soil prior to planting.

The 3 corn diets each contained 35% sorghum (*Sorghum vulgare*) - aerial part with head, ensiled, mature, International Reference No. 3-04-322 (36.7% dry matter), 61% ground corn (88.3% dry matter), and 4% concentrate supplement mixture (90.9% dry matter) on an as-fed basis. These diets were 18% sorghum silage, 77% ground corn, and 5% concentrate supplement mixture on a dry matter basis. The ingredients of the concentrate supplement mixture are listed in Table 11.

Individual animal weights were obtained at the beginning and end of the experiment after an overnight shrink. Group weights were obtained every 28 days during the period. These weights were obtained in order to periodically check on the performance of the steers on each of the 3 diets. Blood, fecal, feed ingredient, and sludge samples were collected each time the steers were weighed. One steer had to be removed from one of the control groups during the course of the experiment due to sickness; the data for that steer were not used in analyses of the results.

The steers were fed *ad libitum* twice daily for a total of 141 days (March 23 to August 11, 1977). The amounts of feed for each diet were recorded at each feeding. The DCS, obtained from a commercial source, was incorporated into the appropriate diet at feeding time. The DCS contained 6.4% moisture.

At the end of the experimental period, the steers were slaughtered at the University of Florida Meats Laboratory, Gainesville. Liver, muscle, and kidney tissues were collected at slaughter. Carcass measurements were obtained.

The DCS, LPS, feed ingredients, blood, fecal, and tissue samples were sent to the Analytical Research Laboratory, Soil Science Department, University of Florida, Gainesville, for metal analyses. Concentrations of selected metals in the sludges and feed ingredients are listed in Table 12.

Analyses of variance for animal performance and carcass quality data were conducted according to the method of Snedecor (1946). The computer package (Statistical Analysis System, North Carolina State University) outlined by Barr *et al.* (1976) was utilized for the statistical analyses of the metal data in blood, feces, and tissues.

RESULTS AND DISCUSSION

Performance and carcass data with beef steers fed 3 experimental diets for a 141 day feeding period are shown in Table 13. Steers fed the LPS corn diet had an average daily gain of 1.04 kg. This compared to an average daily gain of 0.98 and 0.91 kg with steers fed the control corn diet and the diet containing DCS. There were no significant differences among treatments in average daily gain.

Steers fed the LPS corn diet were 8.8 and 15.7% more efficient in converting feed to gain than those fed the control corn and "spiked" corn diets (Table 13). The "spiked" corn diet did not appear unpalatable as evidenced by similar feed intakes.

There were no differences among treatments for any of the carcass quality measurements (Table 13).

The DCS was considerably higher in a number of the metals, Cd, chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), and lead (Pb), than dried LPS (Table 12). The Cd concentration in the concentrate supplement mixture, probably supplied by commercial calcium phosphate, was also higher than expected. Thus, the "spiked" corn diet contained a substantial amount of Cd.

Table 14 shows the concentrations of selected metals in liver and kidney tissues. The Cd, Cu, Fe, and Pb concentrations in livers were higher ($P < 0.01$ for Cd, Cu, and Fe; $P < 0.05$ for Pb) from steers fed the "spiked" corn diet than respective metal concentrations in livers of steers fed the control corn diet or the LPS corn diet. This was contrary to results reported by Bertrand *et al.* (1978), which showed that a high level of DPS in the diet reduced the liver storage of cobalt (Co), Cu, Ni, and zinc (Zn). However, the 2 sludges differed considerably in concentrations and ratios of most of the metals. This may account for the differences in results obtained for the 2 experiments.

The higher ($P < 0.01$) Cd content in livers and kidneys of steers fed the "spiked" corn diet when compared with the other 2 diets was a matter of concern. Ryan (1978) reported that Cd tended to accumulate in visceral organs (liver, kidney, and pancreas). Renal tubular damage was the most important chronic effect of Cd exposure. A high concentration of Cd in the soil can cause an increased concentration of Cd in crops (Chaney and Hornick, 1978). Also, livestock grazing pasture could ingest a sludge adhering to edible plant surfaces. Therefore, the Cd concentration of a sewage sludge could be one of the limiting factors in determining the amount of the material that can be safely applied to agricultural land for the production of food and feed crops.

TABLE 11. CONCENTRATE SUPPLEMENT MIXTURE

	International Ref. No.	Percent
Soybeans (*Glycine max*) - seeds, solvent-extracted, ground, 44%	5-04-604	61.2
Urea - 45% N		11.2
Calcium phosphate - dibasic, commerical	6-01-080	7.5
Limestone - ground, mn 33% calcium	6-02-632	11.2
Trace mineral salt[a]		8.9
Vitamin A palmitate[b]	7-05-143	+

[a] Contained not less than 0.350% Zn, 0.340% Fe, 0.200% Mn, 0.033% Cu, 0.007% I, and 0.005% Co.

[b] Supplied 66,120 IU/kg of concentrate supplement mixture.

TABLE 12. CONCENTRATIONS OF SELECTED METALS IN DRIED CHICAGO DIGESTED SLUDGE, PENSACOLA LIQUID DIGESTED SLUDGE, AND FEED INGREDIENTS IN THE EXPERIMENTAL DIETS

Metals	Chicago sludge	Pensacola liquid sludge[a]	Control corn	LPS corn[b]	Sorghum silage	Concentrate supplement mixture
				$\mu g/g$[c]		
Cd	163	7	<.025	<.025	<.025	1.63
Co	22	10	< .10	< .10	< .10	7.10
Cr	2,888	192	< .10	< .10	< .10	9.63
Cu	1,365	309	1.25	2.50	3.75	19.89
Fe	37,267	3,000	236	214	622	482
Hg	2	5	--[d]	--	--	--
Ni	376	20	< .10	< .10	< .10	7.13
Pb	774	193	< .20	< .20	< .20	2.20
Zn	2,501	2,570	21.5	29.5	23.8	198.5

[a] Solids averaged 3.3%.

[b] Corn produced on soil fertilized with surface applications totaling 19.8 t/ha of Pensacola liquid digested sludge prior to planting.

[c] Dry matter basis.

[d] Not analyzed.

TABLE 13. PERFORMANCE AND CARCASS DATA WITH BEEF STEERS RECEIVING DRIED CHICAGO DIGESTED SLUDGE AND CORN PRODUCED FROM SOIL FERTILIZED WITH PENSACOLA LIQUID DIGESTED SLUDGE IN FEEDLOT DIETS[a]

Item	Control	"Spiked" corn diet[b]	LPS corn diet[c]
Number of animals[d]	7	8	8
Length of trial, days	141	141	141
Average initial weight, kg	292	301	303
Average final weight, kg	430	429	449
Average daily gain, kg	0.98 ± 0.18	0.91 ± 0.07	1.04 ± 0.20
Feed/gain ratio	11.1	11.8	10.2
Average carcass quality grade[e]	17.0 ± 1.3	15.9 ± 1.7	16.1 ± 1.5
Average carcass yield grade	3.4 ± 0.4	2.8 ± 0.5	3.2 ± 0.6

[a] Data presented as means ± SD for 7 or 8 observations where appropriate.

[b] Control corn plus 500 g/head/day of DCS incorporated into the diet.

[c] Diet containing corn produced from soil fertilized with surface applications totaling 19.8 t/ha prior to planting.

[d] Two groups of 4 steers each.

[e] 15 = high good, 16 = low choice, 17 = average choice, 18 = high choice.

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E. FORAGE SORGHUM SILAGES GROWN ON SOIL TREATED WITH LIQUID DIGESTED SLUDGE AND FED TO BEEF STEERS

ABSTRACT

Processed sewage sludges have potential as fertilizers on agricultural land. Forage sorghum (*Sorghum bicolor* (L.) Moench) silages, grown on soil treated with Pensacola liquid digested sludge (LPS) turned under prior to planting, were fed as the main ingredient in the diet of beef steers to determine the effects on animal performance, carcass quality, and concentrations of selected potentially toxic metals in liver, muscle, and kidney tissues. The experimental diets consisted of: (1) control (normal fertilization), (2) LPS #1, i.e., 40.4 metric tons/ha of LPS, and (3) LPS #2, i.e., 110.6 metric tons/ha of LPS. Inclusion of silages grown on soil treated with LPS in the diets had no effect on animal performance and carcass quality measurements of beef steers. The cadmium (Cd) concentrations were lower ($P < 0.05$) in the livers of steers fed the LPS #1 diet. The reason for this was not apparent. However, the concentrations of copper (Cu) and iron (Fe) in livers of steers fed the LPS diets were lower ($P < 0.01$) than those of steers fed the control diet. This indicated that the metals in LPS, even when the material was used as a fertilizer for growing silage, were accumulated to some extent in the sorghum plants and had a detrimental effect at the absorption site and/or at the liver storage site for Cu and Fe. There were no differences among treatments in the concentrations of selected metals in muscle and kidney tissues.

INTRODUCTION

Forage sorghum (*Sorghum bicolor* (L.) Moench) silage, properly supplemented, is a feed with good potential for growing calves or finishing steers in northwest Florida (Bertrand *et al.*, 1974; Bertrand *et al.*, 1975). Sorghum, especially the forage varieties from which two harvests (a first crop and a ratoon crop) per growing season can normally be obtained, can produce substantially more forage per unit of land than corn (*Zea mays* L.) (Dunavin, 1973).

Sludges have potential as a fertilizer on cropland (Hammond, 1974; Lutrick and Bertrand, 1974). Performance was not affected when crops grown on land receiving applications of digested sewage sludge were fed to livestock (Hartman, 1975; USEPA, 1972). However, metal contaminants contained in sludges can accumulate in plant tissues (Chaney *et al.*, 1978). This could be of concern from a human health standpoint when human and industrial wastes are applied to land intended for the production of forage for consumption by food animals (CAST, 1976).

The purpose of this study was to evaluate the effects of forage sorghum silages, grown on soil treated with various levels of Pensacola liquid digested sludge (LPS) prior to planting, in diets of beef steers on performance, carcass quality, and concentrations of selected potentially toxic metals in liver, muscle, and kidney tissues.

MATERIALS AND METHODS

Twenty-four steers (average 178 kg) were weighed and allotted at random from breed groups to 6 experimental groups of 4 steers each. The 6 experimental groups, utilizing 2 groups (replicates) per treatment, were assigned to the 3 following treatments:

1. Control - diet containing forage sorghum silage grown on soil fertilized with 448 kg/ha of 8-24-24 fertilizer at planting time and sidedressed with 224 kg/ha of ammonium nitrate at the first cultivation.

2. LPS #1 - diet containing forage sorghum silage grown on soil treated with surface applications totaling 15 cm/ha (39 metric tons/ha of dry material) of LPS turned under prior to planting.

3. LPS #2 - diet containing forage sorghum silage grown on soil treated with surface applications totaling 22.5 cm/ha (59 metric tons/ha of dry material) of LPS turned under prior to planting.

The feedlot diets consisted of 95% forage sorghum silage (31% dry matter) and 5% concentrate supplement mixture (89% dry matter) on an as-fed basis. These diets were 87% forage sorghum silage and 13% concentrate supplement mixture on a dry matter basis. The ingredients of the concentrate supplement mixture are listed in Table 15.

After an overnight shrink, individual animal weights were obtained at the beginning and end of the experiment. Group weights were obtained every 28 days during the experiment. These weights were obtained in order to periodically check on the performance of the steers on each of the 3 diets. Blood, fecal, and feed ingredient samples were collected each time the steers were weighed.

The trial was initiated on February 22 and terminated on August 9, 1978 (168 days). The steers were fed *ad libitum* once daily. The amounts of feed for each diet were recorded at each feeding.

At the end of the feeding period, the steers were slaughtered at the University of Florida Meats Laboratory, Gainesville. Liver, muscle, and kidney tissues were collected at slaughter. Carcass measurements were obtained by trained personnel at the Meats Laboratory.

The LPS, feed ingredients, blood, fecal, and tissue samples were sent to the Analytical Research Laboratory, University of Florida, Gainesville for metal analyses. Samples were measured by volume (blood) or weight, dried where appropriate (feed ingredients, LPS, and feces), wet-ashed, dissolved in 0.2% nitric acid, and determinations were made with an emission or absorption flame spectrophotometer. Concentrations of selected metals in the sludge and feed ingredients are listed in Table 16.

Analyses of variance for animal performance and carcass quality data were conducted according to the method of Snedecor (1946). The metal concentration data for blood, feces, and tissues (liver, muscle, and kidney) between treatments were tested for significance of variances by the SAS computer method (Statistical Analysis System, North Carolina State University) (Barr *et al.*, 1976).

RESULTS AND DISCUSSION

Performance and carcass data of steers receiving the 3 forage sorghum silage diets for the 168 day period are shown in Table 17. Steers fed the control diet had an average daily gain of 0.55 kg per head, followed by the average daily (0.54 kg/head) of steers fed the LPS #2 diet and the average daily gain (0.45 kg/head) of steers fed the LPS #1 diet. There were no significant differences among treatments in average daily gain. This was probably due to the limited amount of observations and the large variation in individual gain within treatment groups. Steers receiving the control diet were 22.4 and 2.4% more efficient in converting feed to gain than those receiving the LPS #1 and LPS #2 diets, respectively. Feed consumption was not affected by the LPS treatments. The average carcass yield grade was lower ($P < 0.05$) for steers receiving the control diet than that of steers receiving the LPS diets. This was probably due to chance and did not represent true treatment differences. There were no differences among treatments as far as any of the other carcass parameters were concerned.

Concentrations of selected metals in blood and feces are listed in Table 18. The copper (Cu) and iron (Fe) concentrations in blood of steers fed the LPS diets were lower ($P < 0.01$) than those of steers fed the control diet. Earlier studies with dried LPS fed in the diets of feedlot steers also showed slightly lower concentrations of Cu in the blood of steers receiving LPS (Bertrand *et al.*, 1978). Underwood (1971) stated that interactions exist between metals, such as Fe, zinc (Zn), cadmium (Cd), and Cu, which interfere with absorption. Apparently, these metals compete for protein-binding sites in the intestinal mucosa. This may account for the lower concentrations of Cu and Fe in blood of steers consuming forage sorghum silages grown on soil treated with LPS. The Cd, Cu, and Zn concentrations in feces of steers fed the LPS diets were higher ($P < 0.05$ for Cu; $P < 0.01$ for Cd and Zn) than those of steers fed the control diet. These results were not in complete agreement with the concentrations of the metals in the feed ingredients (Table 16).

Concentrations of selected metals in liver and kidney tissues are shown in Table 17. The Cd concentrations were lower ($P < 0.05$) in livers of steers receiving the LPS #1 diet. The reason for this was not apparent. However, the concentrations of Cu and Fe in livers of steers fed the LPS diets were lower ($P < 0.01$) than those of steers fed the control diet.

This agreed with previous studies with steers fed diets containing LPS (Bertrand *et al.*, 1978). This again indicated that the metals in LPS, even when the material was used as a fertilizer for growing the silages, were accumulated to some extent in the sorghum plants and had a detrimental effect at the absorption site and/or at the liver storage site for Cu and Fe. There were no differences among treatments in the concentrations of the selected metals in kidney tissues (Table 19).

TABLE 14. CONCENTRATIONS OF SELECTED METALS IN LIVER AND MUSCLE TISSUES[a]

	Control corn diet	"Spiked" corn diet[b]	LPS corn diet[c]
Number of observations	7	8	8
Metals in liver tissues	- - - - - - - - - - - - - -	$\mu g/g^{d}$	- - - - - - - - - - - - -
Cd	$\leq .07^{g} \pm 0.03$	$3.50^{h} \pm 1.10$	$\leq .05^{g} \pm 0.01$
Cu	$16.61^{g} \pm 8.39$	$40.72^{h} \pm 20.77$	$18.09^{g} \pm 3.62$
Fe	$87.63^{g} \pm 19.50$	$262.66^{h} \pm 135.61$	$93.81^{g} \pm 18.37$
Pb	$\leq .10^{e}$	$\leq .26^{f} \pm 0.24$	$\leq .10^{e}$
Metals in kidney tissues			
Cd	$0.27^{g} \pm 0.06$	$13.81^{h} \pm 3.29$	$0.33^{g} \pm 0.11$
Cu	4.59 ± 0.86	4.98 ± 0.64	4.59 ± 0.46
Fe	$81.03^{g} \pm 15.20$	$131.25^{h} \pm 28.33$	$77.28^{g} \pm 6.57$
Pb	$< .10^{g}$	$0.72^{h} \pm 0.22$	$\leq .13^{g} \pm 0.08$

[a] Data presented as means ± SD where appropriate.

[b] Control corn plus 500 g/head/day of DCS incorporated into the diet.

[c] Diet containing corn produced from soil fertilized with surface applications totaling 19.8 t/ha.

[d] Fresh tissue basis.

Table 14. (Continued)

[e,f] Means in a row with different superscripts differ significantly ($P < 0.05$).

[g,h] Means in a row with different superscripts differ significantly ($P < 0.01$).

TABLE 15. COMPOSITION OF THE CONCENTRATE SUPPLEMENT MIXTURE

Ingredients	Percentage
Soybean meal (44% protein)	82.3
Urea - 45% N	6.4
Dicalcium/monocalcium phosphate	7.5
Salt (trace-mineralized)[a]	3.8
Vitamin A supplement[b]	+

[a] Contained not less than 0.350% Zn, 0.340% Fe, 0.200% Mn, 0.033% Cu, 0.007% I, and 0.005% Co.

[b] Rovimix A-650 (vitamin A supplement containing 650,000 IU/g) added at the level of 22.05 million IU/metric ton or 22,050 IU/kg of concentrate supplement mixture.

TABLE 16. CONCENTRATIONS OF SELECTED METALS IN PENSACOLA LIQUID DIGESTED SLUDGE AND FEED INGREDIENTS IN THE EXPERIMENTAL DIETS

Metals	Pensacola sludge[a]	Control silage	LPS #1 silage[b]	LPS #2 silage[c]	Concentrate supplement mixture
	- - - - - - - -	- - - - - - - -	(μg/g dry wt)	- - - - - - - -	- - - - - - - -
Cd	12	≤ 0.17	0.25	0.31	0.38
Co	68	≤ 0.99	1.56	< 0.10	2.19
Cr	282	2.19	≤ 2.21	≤ 0.68	2.81
Cu	587	9.94	7.88	5.82	14.25
Fe	5,416	782	1,188	1,019	641
Ni	51	2.19	≤ 0.39	≤ 0.39	3.75
Pb	526	≤ 0.39	< 0.10	≤ 0.39	≤ 0.39
Zn	3,393	63.13	47.81	79.06	113.13

[a] Solids averaged 2.6%

[b] Forage sorghum silage grown on soil treated with surface applications totaling 15 cm/ha of Pensacola liquid digested sludge prior to planting. Each feed ingredient mean was based on 4 observations.

[c] Forage sorghum silage grown on soil treated with surface applications totaling 22.5 cm/ha of Pensacola liquid digested sludge prior to planting.

TABLE 17. PERFORMANCE AND CARCASS DATA OF BEEF STEERS RECEIVING DIETS CONTAINING FORAGE SORGHUM SILAGES GROWN ON SOIL TREATED WITH VARIOUS LEVELS OF PENSACOLA LIQUID DIGESTED SLUDGE PRIOR TO PLANTING[a]

Item	Control diet	LPS #1 diet[b]	LPS #2 diet[c]
Number of animals	8[d]	8	8
Length of trial, days	168	168	168
Average initial wt, kg	176	179	179
Average final wt, kg	269	254	269
Average daily gain, kg	0.55 ± 0.18	0.45 ± 0.08	0.54 ± 0.06
Feed/gain ratio	28.6	35.0	29.3
Average carcass yield grade	$1.6^e \pm 0.2$	$1.9^f \pm 0.1$	$1.8^f \pm 0.2$
Average dressing percent	53.2 ± 1.8	53.1 ± 1.6	53.9 ± 4.7

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[a] Data presented as means ± SD where appropriate for 8 observations.

[b] Diet containing forage sorghum silage grown on soil treated with surface applications totaling 15 cm/ha of LPS prior to planting.

[c] Diet containing forage sorghum silage grown on soil treated with surface applications totaling 22.5 cm/ha LPS prior to planting.

[d] Two groups of 4 steers each.

[e,f] Means in a row with different superscripts differ significantly ($P < 0.05$).

TABLE 18. CONCENTRATIONS OF SELECTED METALS IN BLOOD AND FECES[a]

Metals	Treatments		
	Control diet	LPS #1 diet[b]	LPS #2 diet[c]
No. of observations	40	40	40
	(Blood, g/ml)		
Cu	0.82^{g} ± 0.14	0.52^{f} ± 0.22	0.59^{f} ± 0.23
Fe	482^{g} ± 65	$458^{f,g}$ ± 76	419^{f} ± 62
Pb	< 0.10	< 0.10	< 0.10
Zn	4.18 ± 0.51	3.91 ± 0.61	3.96 ± 0.92
No. of observations	34	33	31
	(Feces, g/g dry wt)		
Cd	0.29^{f} ± 0.19	0.56^{g} ± 0.28	0.54^{g} ± 0.19
Co	1.00 ± 1.06	1.32 ± 0.80	1.78 ± 1.59
Cr	4.58 ± 2.97	4.02 ± 1.55	3.77 ± 1.73
Cu	14.57^{d} ± 7.52	16.28^{e} ± 4.63	17.11^{e} ± 5.12
Fe	2,947 ± 860	2,994 ± 823	2,705 ± 957
Pb	2.27 ± 1.35	1.88 ± 1.44	1.84 ± 1.96
Zn	96.9^{f} ± 33.0	205.4^{g} ± 54.0	224.5^{g} ± 90.5

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[a] Data presented as means ± SD where appropriate.

TABLE 18. Continued

[b] Diet containing forage sorghum silage grown on soil treated with surface applications totaling 15 cm/ha of LPS prior to planting.

[c] Diet containing forage sorghum silage grown on soil treated with surface applications totaling 22.5 cm/ha of LPS prior to planting.

[d,e] Means in a row with different superscripts differ significantly ($P < 0.05$).

[f,g] Means in a row with different superscripts differ significantly ($P < 0.01$).

TABLE 19. CONCENTRATIONS OF SELECTED METALS IN LIVER AND KIDNEY TISSUES[a]

Metals	Treatments		
	Control diet	LPS #1 diet[b]	LPS #2 diet[c]
No. of observations	8	8	8
	- - - - - - -	(Liver, μg/g wet wt)	- - - - - - - -
Cd	0.12^{e} ± 0.01	0.08^{d} ± 0.01	0.11^{e} ± 0.03
Cu	8.61^{g} ± 6.27	2.11^{f} ± 0.42	3.14^{f} ± 1.61
Fe	368^{g} ± 111	350^{g} ± 120	185^{f} ± 73
	- - - - - - -	(Kidney, μg/g wet wt)	- - - - - - - -
Cd	0.46 ± 0.18	0.30 ± 0.11	0.40 ± 0.24
Cu	3.23 ± 0.32	2.90 ± 0.35	3.20 ± 0.60
Fe	113.9 ± 11.0	96.9 ± 31.5	89.3 ± 17.7
Ni	17.9 ± 1.6	18.0 ± 1.8	17.6 ± 1.3

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[a] Data presented as means ± SD where appropriate for 8 observations.

[b] Diet containing forage sorghum silage grown on soil treated with surface applications totaling 15 cm/ha of LPS prior to planting.

[c] Diet containing forage sorghum silage grown on soil treated with surface applications totaling 22.5 cm/ha of LPS prior to planting.

[d,e] Means in a row with different superscripts differ significantly ($P < 0.05$).

[f,g] Means in a row with different superscripts differ significantly ($P < 0.01$).

F. PENSACOLA BAHIAGRASS PASTURES FERTILIZED WITH PENSACOLA LIQUID DIGESTED SLUDGE AND GRAZED BY BEEF STEERS

ABSTRACT

Forty-eight beef steers were randomly allotted and grazed 1 of 3 Pensacola bahiagrass pastures for 168 days to determine the effects of pasture treatments with Pensacola liquid digested sludge (LPS) on animal performance, carcass quality, and concentrations of selected potentially toxic metals in liver, muscle, and kidney tissues. The experimental pastures consisted of: (1) control, i.e., normal fertilization, (2) LPS #1, i.e., a total of 16 metric tons/ha of LPS, and (3) LPS #2, i.e., a total of 32 metric tons/ha of LPS. There were no differences among treatments for any of the animal performance and carcass parameters. The copper (Cu) and iron (Fe) concentrations in blood of steers grazing the LPS pastures were lower ($P < 0.05$) than those of steers grazing control pastures. Significant differences existed among treatments with respect to most of the metals in fecal material. Metal concentrations in fecal material increased as the level of LPS applied to pasture was increased. The Cu concentrations in liver tissues were lower ($P < 0.01$) for steers grazing the LPS pastures. There were no differences among treatments in the concentrations of selected metals in muscle tissues. The estimated sludge content on the grazed forages for the LPS #1 and LPS #2 pastures was 2.21 and 4.74%, respectively.

INTRODUCTION

Warm-season perennial grass pastures, such as Pensacola bahiagrass (*Paspalum notatum*, Flugge), provide a long grazing period and relatively good gains with long yearlings in northwest Florida (Bertrand and Dunavin, 1968). These pastures appear to be an ideal year-round site for the disposal of processed sewage sludges.

Sludges have a potential as a fertilizer on cropland (Hammond, 1974; Lutrick and Bertrand, 1974). Cattle grazing sludge-treated pastures could ingest metal contaminants contained in the sludges and accumulated in plant tissues or sludge adhering to edible plant surfaces (Chaney *et al.*, 1978; Fitzgerald, 1977). This could be of concern from a human health standpoint when human and industrial wastes are applied on pasture land intended for grazing by food animals (CAST, 1976). However, Kienholz *et al.* (1976), reported that aged cows grazed sludge-treated pastures for 5 years and found no marked differences in heavy metal concentrations in their tissues when compared to control cows.

The purpose of this study was to determine the effects of Pensacola liquid digested sludge (LPS) applied to Pensacola bahiagrass pastures grazed with beef steers on animal performance, carcass quality, and concentrations of selected potentially toxic metals in liver, muscle, and kidney tissues.

EXPERIMENTAL PROCEDURE

Forty-eight beef steers (average 289 kg) of mixed breeding were weighed and allotted at random to 6 experimental groups of 8 steers each. The 6 experimental groups, utilizing 2 groups (replicates) per treatment, were assigned to 3 pasture treatments as follows: (1) control - rotational grazing of Pensacola bahiagrass pastures treated with 280 kg/ha of 8-24-24 fertilizer prior to grazing and 5 applications of 112 kg/ha each of ammonium nitrate during the grazing season, (2) LPS #1 - same pastures as above treated with 7.5 cm/ha (16 metric tons/ha of dry material) of LPS applied at the rate of 3.75 cm/ha during the winter prior to grazing and 3.75 cm/ha during the grazing season, along with 112 kg/ha of muriate of potash prior to grazing, and (3) LPS #2 - same pastures as above treated with 15 cm/ha (32 metric tons/ha of dry material) of LPS applied at the rate of 7.5 cm/ha during the winter prior to grazing and 7.5 cm/ha during the grazing season, along with 112 kg/ha of muriate of potash prior to grazing.

Each pasture replicate was subdivided into 2 1.0 ha plots which were grazed rotationally by 8 steers initially. The trial was initiated on May 23, 1979, and terminated on November 7, 1979 (168 days).

After an overnight shrink, individual animal weights were obtained at the beginning and end of the trial period. Group weights were obtained every 28 days during the experiment to periodically check on performance of the steers on each of the 3 pasture treatments. Blood, fecal, forage, and LPS samples were collected each time the steers were weighed. The blood and fecal samples were collected from the same 4 steers each time from each experimental group of 8 steers. Additional grazer animals of the same type and size were added and removed as needed to keep the forages uniformly grazed. Each experimental group of steers was rotated between the 2 pasture plots assigned to it as required for best utilization of good quality forage.

The LPS was applied with a Calumet sludge spreader initially during the winter and during the grazing season on the LPS pasture plots as soon as possible after the steers were rotated to the other rotational plots. Each LPS application on the soil and forage was performed at the rate of 0.83 cm/ha. Certain proximate components for each of the 3 Pensacola bahiagrass forages are shown in Table 20.

At the end of the experimental period, the steers were slaughtered at the University of Florida Meats Laboratory, Gainesville. Liver, muscle, and kidney tissues from the 4 selected steers in each experimental group were collected at slaughter. Carcass measurements were obtained by trained personnel at the Meats Laboratory.

The LPS, forage, blood, fecal, and tissue samples were sent to the Analytical Research Laboratory, Soil Science Department, University of Florida, Gainesville for metal analyses. Samples were measured by volume (blood) or weight, dried where appropriate (forages and feces), wet-ashed, dissolved in 0.2% nitric acid and determinations were made with an emission or absorption flame spectrophotometer. Concentrations of selected metals in LPS and forages are listed in Table 21.

Analyses of variance for animal performance and carcass quality data were conducted according to the method of Snedecor (1946). The metal concentration data for blood, feces, and tissues (liver, muscle, and kidney) between treatments were tested for significance of variance by the SAS computer method (Statistical Analysis System, North Carolina State University) outlined by Barr *et al.* (1976).

The estimated sludge content on the grazed forages was calculated by the method of Decker *et al.* (1977).

RESULTS AND DISCUSSION

Performance and carcass data of beef steers grazing the Pensacola bahiagrass pastures for the 168 day period are shown in Table 22. Steers grazing pastures receiving the 3 treatments had identical average daily gains (0.30 kg). However, steers grazing the LPS pastures had more available forage and higher stocking rates than those grazing the control pastures and thus had higher gains per hectare (235 kg for LPS #1 and LPS #2 versus 199 kg for control). There were no differences among treatments for any of the animal performance and carcass parameters.

Concentrations of selected metals in blood and feces are listed in Table 23. The copper (Cu) and iron (Fe) concentrations in blood of steers grazing the LPS pastures were lower ($P < 0.05$) than those of steers grazing control pastures. Earlier studies with DPS fed in the diets of feedlot steers also showed slightly lower concentrations of Cu in the blood of steers receiving DPS (Bertrand *et al.*, 1978). Underwood (1971) stated that interactions exist between metals, such as Fe, zinc (Zn), cadmium (Cd), Cu and others, which interfere with absorption. Apparently, these metals compete for protein-binding sites in the intestinal mucosa. This may account for the lower concentrations of Cu and Fe in blood of steers grazing the forages treated with LPS.

Significant differences existed among treatments with respect to most of the metals in fecal material (Table 23). Metal concentrations in fecal material increased as levels of LPS applied to pastures were increased. The data indicated that excretion in the feces played an important part in the elimination of excessive amounts of metals in the diet. This agreed with the results obtained by Bertrand *et al.* (1978) where various levels of DPS were added to feedlot diets.

Concentrations of selected metals in liver, muscle, and kidney tissues are shown in Table 24. The Cd concentrations were higher ($P < 0.05$) in livers of steers grazing the LPS pastures when compared to those obtained

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with steers grazing the control pastures. Bertrand *et al.* (1980) found high accumulations of Cd in both liver and kidney tissues of steers fed a sludge high in Cd. The Cu concentrations in liver tissues were lower ($P < 0.01$) for steers grazing the LPS pastures. This suggested that the other metals contained in the LPS may have a detrimental effect at the absorption site and/or at the liver storage site for Cu. The nickel (Ni) concentrations were higher ($P < 0.05$) in liver tissues of steers grazing the LPS #1 pastures.

There were no differences among treatments in the concentrations of selected metals in muscle tissues (Table 24). The Ni concentrations were higher ($P < 0.05$) in kidney tissues of steers grazing the LPS #1 pastures. The reason for higher concentrations of Ni in both liver and kidney tissues of steers grazing the LPS #1 pastures was not clear.

The estimated sludge content on the grazed forages on a dry matter basis is shown in Table 25. The estimated sludge content of 2.21% on the grazed forages for the LPS #1 pastures agreed very closely with the estimate of 2.18% by Decker *et al.* (1977); while the estimated sludge content of 4.74% on the grazed forages for the LPS #2 pastures was somewhat higher.

TABLE 20. CERTAIN PROXIMATE COMPONENTS OF THE PENSACOLA BAHIAGRASS FORAGES[a]

Proximate components	Control	LPS #1[b]	LPS #2[c]
Dry matter, %	32.98	32.16	29.01
Crude protein, %	4.07	4.68	4.89
Ash, %	1.78	2.27	2.71
Crude fiber, %	9.76	9.06	7.43

[a] Means of 14 samples collected at intervals during the trial period.

[b] A total of 16 metric tons/ha of Pensacola liquid digested sludge applied prior to and during the grazing season.

[c] A total of 32 metric tons/ha of Pensacola liquid digested sludge applied prior to and during the grazing season.

TABLE 21. CONCENTRATIONS OF SELECTED METALS IN PENSACOLA LIQUID DIGESTED SLUDGE AND PENSACOLA BAHIAGRASS FORAGES

Metals	Pensacola Liquid Sludge[a]	Control forage	LPS #1 forage[b]	LPS #2 forage[c]
	- - - - - - - - - - - (g/g dry wt)	- - - - - - - - - -		
Cd	≤ 7	≤ .15	0.36	0.78
Co	≤ .10	≤ .38	≤ .38	≤ .66
Cr	256	2.68	6.11	9.56
Cu	473	6.30	19.00	31.74
Fe	11,838	101	466	651
Ni	93	1.95	2.24	2.70
Pb	397	≤ .75	11.03	20.48
Zn	2,083	27.49	79.56	145.50

[a] Solids averaged 2.14%.

[b] A total of 16 metric tons/ha of Pensacola liquid digested sludge applied prior to and during the grazing season.

[c] A total of 32 metric tons/ha of Pensacola liquid digested sludge applied prior to and during the grazing season.

TABLE 22. PERFORMANCE AND CARCASS DATA OF BEEF STEERS GRAZING PENSACOLA BAHIAGRASS PASTURES TREATED WITH PENSACOLA LIQUID DIGESTED SLUDGE[a]

	Treatments		
	Control	LPS #1[b]	LPS #2[c]
Initial no. of animals	16[d]	16	16
Length of grazing, days	168	168	168
Average initial wt, kg	291	286	289
Average final wt, kg	341	336	340
Average gain/animal, kg	50	50	51
Average daily gain, kg	0.30 ± 0.12	0.30 ± 0.13	0.30 ± 0.09
Animal days/ha[e]	664	783	783
Stocking rate/ha[e]	4.0	4.7	4.7
Gain/ha, kg	199	235	235
Gain/ha/day, kg	1.18	1.40	1.40
Average carcass quality grade	11.18 ± 1.7	11.8 ± 1.1	11.9 ± 1.3
Average carcass yield grade	2.1 ± 0.5	1.9 ± 0.5	1.8 ± 0.5
Average hot carcass wt, kg	176	176	177
Average dressing percentage	51.6 ± 2.6	52.4 ± 2.5	52.1 ± 3.4

TABLE 22. Continued

[a] Data presented as means ± SD where appropriate.

[b] A total of 16 metric tons/ha of Pensacola liquid digested sludge applied prior to and during the grazing season.

[c] A total of 32 metric tons/ha of Pensacola liquid digested sludge applied prior to and during the grazing season.

[d] Two experimental groups of 8 steers each.

[e] Additional grazer animals of the same type and size were added and removed as needed to keep the forage uniformly grazed.

TABLE 23. CONCENTRATIONS OF SELECTED METALS IN BLOOD AND FECES[a]

	Treaments		
Metals	Control	LPS #1[b]	LPS #2[c]
No. of observations	48	48	48
		(Blood, µg/ml)	
Cd	<.01	<.01	<.01
Co	<.10	<.10	<.10
Cr	0.55 ± 0.12	0.50 ± 0.15	0.53 ± 0.14
Cu	0.84[e] ± 0.10	0.73[d] ± 0.23	0.76[d] ± 0.13
Fe	514[e] ± 78	491[d,e] ± 69	478[d] ± 89
Ni	<.10	<.10	<.10
Pb	<.10	<.10	<.10
Zn	3.74 ± 0.94	3.95 ± 0.62	3.79 ± 1.05
No. of observations	41	44	44
		(Feces, µg/ dry wt)	
Cd	≤0.12[f] ± 0.21	0.96[g] ± 0.76	1.35[h] ± 1.04
Co	≤ 0.53 ± 0.68	≤0.76 ± 0.99	≤0.71 ± 0.91
Cr	3.79[f] ± 3.15	9.90[g] ± 6.90	11.44[h] ± 10.12
Cu	13.43[f] ± 5.18	32.14[g] ± 19.77	38.15[h] ± 29.85
Fe	841[d] ± 551	1,129[e] ± 667	1,142[e] ± 843
Ni	2.15[f] ± 2.22	4.26[g] ± 2.28	4.39[g] ± 2.51
Pb	3.86[f] ± 4.61	20.92[g] ± 21.36	23.26[g] ± 23.03
Zn	70.97 ± 33.54	234.35[g] ± 117.80	288.53[h] ± 177.82

TABLE 23. Continued

[a] Data presented as means ± SD where appropriate.

[b] A total of 16 metric tons/ha of Pensacola liquid digested sludge applied prior to and during the grazing season.

[c] A total of 32 metric tons/ha of Pensacola liquid digested sludge applied prior to and during the grazing season.

[d,e] Means in a row with different superscripts differ significantly ($P < 0.05$).

[f,g,h] Means in a row with different superscripts differ significantly ($P < 0.01$).

TABLE 24. CONCENTRATIONS OF SELECTED METALS IN LIVER, MUSCLE, AND KIDNEY TISSUES[a]

	Treatments		
Metals	Controls	LPS #1[b]	LPS #2[c]
No. of observations	8	8	8
	- - - - - - - - - (Liver, µg/g wet wt) - - - - - - - - -		
Cd	0.04[d] ± 0.01	0.05[e] ± 0.02	0.07[e] ± 0.03
Co	<.10	< .10	< .10
Cr	0.73[e] ± 0.02	0.72[e] ± 0.01	0.71[d] ± 0.01
Cu	21.73[g] ± 12.71	6.91[f] ± 5.11	6.31[f] ± 4.12
Fe	82.28 ± 23.80	116.13 ± 29.42	125.88 ± 53.11
Ni	≤0.18[d] ± 0.21	≤0.56[e] ± 0.25	< .10[d]
Pb	≤ 0.32 ± 0.62	≤ 0.31 ± 0.60	≤ 0.49 ± 1.11
Zn	38.48 ± 5.30	38.64 ± 6.96	39.50 ± 4.52
	- - - - - - - (Muscle, µg/g wet wt) - - - - - - -		
Cd	< .01	< .01	< .01
Co	< .10	< .10	< .10
Cr	0.73 ± 0.01	0.74 ± 0.03	0.73 ± 0.02
Cu	1.03 ± 0.15	0.96 ± 0.14	0.96 ± 0.11
Fe	39.18 ± 8.33	39.45 ± 5.21	48.54 ± 6.82
Ni	≤ 0.37 ± 0.47	≤ 0.58 ± 0.36	< .10
Pb	≤ .10	≤ 0.74 ± 1.36	≤ 0.62 ± 1.11
Zn	45.15 ± 8.70	48.88 ± 12.68	53.48 ± 7.91

TABLE 24. Continued

	Treatments		
	Control	LPS #1[b]	LPS #2[c]
No. of observations	8	8	8
	------	(Kidney, g/g wet wt)	-------
Cd	0.27 ± 0.10	0.28 ± 0.27	0.24 ± 0.08
Co	< .10	< .10	< .10
Cr	0.73 ± 0.02	0.73 ± 0.02	0.73 ± 0.01
Cu	3.91 ± 0.35	3.76 ± 0.36	3.71 ± 0.31
Fe	87.26 ± 22.06	88.90 ± 18.09	84.21 ± 26.86
Ni	≤ 0.22[d] ± 0.23	0.64[e] ± 0.17	< .10[d]
Pb	≤ 1.83 ± 3.24	≤ 1.89 ± 2.02	≤ 0.76 ± 1.87
Zn	22.08 ± 2.20	20.66 ± 2.46	20.19 ± 1.12

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[a] Data presented as means ± SD where appropriate.

[b] A total of 22 metric tons/ha of Pensacola liquid digested sludge applied prior to and during the grazing season.

[c] A total of 44 metric tons/ha of Pensacola liquid digested sludge applied prior to and during the grazing season.

[d,e] Means in a row with different superscripts differ significantly ($P < 0.05$).

[f,g] Means in a row with different superscripts differ significantly ($P < 0.01$).

TABLE 25. ESTIMATION OF SLUDGE CONTENT ON GRAZED FORAGES[a]

Metals	Control forage[b]	LPS #1 forage[b]	LPS #2 forage[b]	Difference LPS#1	Difference LPS #2	Pensacola sludge[b]	Sludge on forage LPS #1	Sludge on forage LPS #2
	- - - - - -	- - - - - -	- - - - - -	(µg/g dry wt)		- - - - - -	- - - (%)	- - -
Cd	≤ 0.15	0.36	0.78	0.21	0.63	7	3.00	9.00
Co	≤ 0.38	≤ 0.38	≤ 0.66	-[c]	-[c]	.10	--	--
Cr	2.68	6.11	9.56	3.43	6.88	256	1.34	2.69
Cu	6.30	19.00	31.74	12.70	25.44	473	2.68	5.38
Fe	101	466	651	365	550	11,838	3.08	4.65
Ni	1.95	2.24	2.70	0.29	0.75	93	0.31	0.81
Pb	≤ 0.75	11.03	20.48	10.28	19.73	397	2.59	4.97
Zn	27.49	79.56	145.50	52.07	118.01	2,083	2.50	5.67
Means							2.21	4.74

[a] Dry matter basis.

[b] Taken from Table 21.

[c] Not used in estimation.

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CATTLE - OTHER HEALTH EFFECTS

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Summary of Results - 1975-1980

CATTLE - OTHER HEALTH EFFECTS

G. T. Edds, C. F. Simpson, O. Osuna, K. E. Ferslew,
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The 1975 preliminary trial of mixing 100 g dried Pensacola sludge (DPS) daily in the ration for 3 steers versus a regular ration for 3 control animals was utilized to determine which health parameters should be monitored for future experiments. The cattle were bled monthly as well as fecals samples taken to examine for level of parasite infection. The hematocrit, red and white blood cell counts, sedimentation rates, and parasite egg counts were determined. Differential white cell counts and prothrombin times were done. Pasture grass samples were secured for cyanide and bacteriological assays. There were no significant differences in any of the parameters measured.

The following year (1976), 24 steers were secured and assigned to 3 groups, and allowed to graze on winter annual pastures. A control group (no treatment) and 2 groups grazed on pastures pretreated with liquid Pensacola sludge (LPS) during the trial. Blood and fecal samples were secured from each animal at the outset and at 28 day intervals during the trial. The following parameters were followed: hematocrits, red and white cell counts, prothrombin times, differential counts, and parasite egg counts. Four representative animals from each group were slaughtered after 5 months. There were no significant differences in growth performance nor in the measured health parameters.

In August, 1976, in a 120 day feeding trial, another group of 24 steers were allotted to 3 groups, a control group receiving a regular feedlot ration, one group received this ration plus 250 g dried Pensacola sludge (DPS) mixed in their feed, and the third group received a ration containing 500 g DPS daily in their feed. Parameters monitored at the outset and monthly included red and white cell counts, prothrombin times, and parasite egg counts. Parasite egg counts were initially high for trichostrongyles, neoascarids, and coccidia. The feed samples collected were also examined for presence of mycotoxins. Samples of the DPS were collected for pesticide assays and samples of the blood, feces, sludge, and feed provided to bacteriology for examination. Blood samples from each animal were examined for serum enzyme levels including alkaline phosphatase, AP, gamma-glytamyl transpeptidase (γGT), serum glutamate oxaloacetate transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT). Finally, at slaughter, liver, kidney, and muscle tissues were collected and examined for pathologic changes.

There were no significant differences in growth performance between the control and 250 g/head/day groups, but there was significant growth suppression in the growth of the group receiving 500 g daily and there were significant increases in the AP, γGT, SGOT, and SGPT levels in the groups of steers on the 250 and 500 g/head/day after 8, 12, and 16 weeks. In addition, there was a significant increase in the cadmium level of kidney tissues collected at slaughter in the animals receiving 500 g DPS daily.

An experiment, begun in March 1977, included 3 groups of steers, controls received a ration containing corn from soils treated with regular fertilizers, one group received a ration including corn from soil pretreated with 7.5 cm/hectare liquid Pensacola slidge (LPS), and a third group received the same ration as the controls but also received 500 g/head/day of dried Chicago sludge (DCS) for 5 months. Analysis of variance showed that γGT and glutamic oxaloacetic transaminase (GOT) levels were significantly increased for the steers receiving 500 g DCS daily. Likewise, the steers receiving corn from soil pretreated with LPS showed increased in GOT, glutamic pyruvic transaminase (GPT) and white blood cell counts (Table 1).

Clinical interpretation of these values from the "treated" steers suggest that parenchymal liver disease was present in both groups resulting from heavy metal intoxication or parasitic invasion by flukes. Liver fluke ova were observed in fecal samples from all 3 groups; the 7.5 cm/ha corn group had a greater number of animals affected (5/7) than the DCS group (2/7). The liver sections secured at slaughter of the latter group also displayed biliary obstruction and icterus. The control corn, 7.5 cm/ha LPS corn and sorghum silage were examined for aflatoxin B_1 and found to be negative. Animals receiving the 3 rations were negative to tetracyclines and sulfonamides.

A steer feeding trial was initiated in 1978 consisting of a control group, one group consumed forage sorghum silage pretreated with 15 cm/ha, and a third group consumed silage from soil pretreated with 22.5 cm/ha LPS. Blood and fecal samples were collected for metals, pesticides, and drug residue studies; small sections of each were fixed in formalin for histopathologic studies. Fecal examination showed the 3 groups of animals were moderately parasitized with trichostrongyles, coccidia, and monezia.

The serum enzyme levels for AP, γGT, GOT, and GPT remained consistent throughout the trial although there was some anemia as the trial progressed in the group receiving forage silage from soils pretreated at the 22.5 cm/ha.

Kidney and livers were collected from these steers, frozen, and utilized in mouse feeding trials.

The final EPA-sponsored cattle trial at Jay included 3 groups of cattle fed on bahiagrass pastures which had received 0, 7.5 cm/ha LPS before grazing and 7.5 cm/ha during grazing, and the third pastures had received 15 cm/ha before and 15 cm/ha during grazing. Blood was collected at the start and monthly during the experiments to determine red and white cell counts, packed cell volume, hemoglobin levels, serum enzyme levels for AP, γGT, GOT, and GPT. Feces was secured to evaluate the parasite levels. Split samples were also provided to the bacteriology and pesticide laboratories for analysis.

Cattle in the 2 groups fed on the treated pastures developed significant decreases in the packed cell volumes and numbers of red blood cells present. Finding of an increased incidence of sarcocysts in the cardiac and skeletal muscles of the cattle in the groups feeding on the LPS treated pastures suggested that the sludge was contaminated at the time of application. This may pose a public health hazard to humans consuming meat from such exposed and infected animals. The developing anemia may also have been associated with the sarcocyst infection in the steers.

TABLE 1. STATISTICAL ANALYSES OF CLINICAL CHEMISTRY AND HEMATOLOGIC PROFILES IN BEEF CATTLE (JAY) FED DCS OR CORN[1] FROM DCS TREATED SOIL

Parameter	Group	Correlation to Control[2]	t-test (α)
γGT	500 g/head/day or corn	↑	0.05
GOT	500 g/head/day or corn	↑	0.025
GPT	500 g/head/day or corn	↑	0.05
WBC	500 g/head/day or corn	↑	0.05

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[1] ↑ - above the normal mean

↓ - below the normal mean

[2] Soil supplemented with 8 t/ha

THE EFFECTS OF RECYCLED CATTLE MANURE ON THE HEALTH AND PERFORMANCE OF STEERS

M. F. Richter

METHODS

Two 1-year feeding trials have been completed. In each trial, 20 animals were divided into 5 groups of 4 animals: group 1, initial slaughter group; group 2, control ration; group 3, manure silage, no withdrawal; group 4, manure silage, 10 days withdrawal; group 5, manure silage, 20 days withdrawal. All animals were adapted to the feed and feeding facilities before the start of the trial. At the beginning of the trial, the initial slaughter group was sacrificed for baseline data on the body composition of the animals. The remaining 16 animals were placed on treatment for approximately 200 days. Manure was withdrawn from the rations of 2 of the groups 10 and 20 days before slaughter. The control ration contained corn grain, citrus pulp, cottonseed meal, pelleted bagasse, malasses, and minerals. The manure ration contained ensiled cattle manure at a level such that 20% of the dry matter of the ration was from manure. The silage was made by mixing raw cattle manure with pelleted bagasse at a 60:40 ratio resulting in a dry matter of 50%. At slaughter, liver, muscle, and kidney samples were taken and analyses were conducted for drugs, pesticides, and heavy metals.

RESULTS

Animals on the control ration consumed an average of 7.1 kg of feed per day and gained an average of .85 kg per day; manure fed animals consumed an average of 10.7 kg of feed per day and gained .76 kg per day.

The control animals consumed 6.1 kg of dry matter; the manure fed animals, 6.4 kg, of which 4.4 kg was concentrate feed identical to the control ration. The feed conversion (kg feed dry matter/kg gain) was 7.2 for the control animals and 8.4 for the manure fed animals. However, if only concentrate feed dry matter is considered, the concentrate feed conversion for the manure fed animals is 5.8. Therefore, the manure silage significantly contributed to the performance of animals consuming it ($P < 0.05$). The manure fed steers gained 89% as much as the control animals while consuming only 72% as much concentrate feed.

The results of the first metabolism trial show that the manure silage significantly ($P < 0.01$) reduced the digestibility of all the proximate components. Digestibility of manure and bagasse are both low so this was expected. The results are tabulated as follows:

DIGESTIBILITY AND NITROGEN BALANCE (4 ANIMALS/TREATMENT)

	Control ration	Manure silage ration
Dry matter, %	82.7 ± 3.2	64.7 ± 0.92
Crude protein, %	70.5 ± 6.6	56.0 ± 2.4
Crude fiber, %	66.7 ± 8.9	41.3 ± 2.6
Nitrogen free extract, %	88.7 ± 2.2	74.2 ± 1.1
Ether extract, %	84.8 ± 5.8	51.9 ± 22.2
Organic matter, %	84.2 ± 3.3	66.9 + 1.3
Nitrogen balance, g/day	7.2 ± 11.1	2.6 ± 48.7

The nitrogen balance was not different between the 2 rations and indicated that the animals were in positive balance. This was expected since the animals gained weight on both treatments. These data show that the manure silage has a feeding value approximately equal to hay.

The tissue concentrations of copper, chrome, cadmium, zinc, cobalt, and nickel was not increased due to feeding manure silage. In addition, there was no net effect due to withdrawal of the manure silage prior to slaughter for either 10 or 20 days. The tissue levels of selected minerals are presented in Table 1.

Mineral balances were measured during the metabolism trial. These indicated no significant differences existed between animals on control and the manure silage rations. Additional carcass examinations showed no pathologic lesions, bacterial contamination or other differences which would indicate adverse health or meat safety effects of the manure silage ration.

CONCLUSIONS

Based on data from the first year's study, the feeding of the manure silage did not adversely effect the health or the food safety of the animals; performance will be reduced slightly on this type of ration due to its lower energy content; feeding value of the manure silage appears to be equal to a good quality hay, and finally, feed savings are achieved since less concentrate feed is necessary per pound of gain.

TABLE 1. TISSUE LEVELS OF SELECTED MINERALS, 1978

4 animals/group M = muscle K = kidney L = liver		ppm, fresh tissue ± SD					
		Cu	Cr	Cd	Zn	Co	Ni
Initial slaughter	M	0.78 ± 0.15	0.19 ± 0.04	.04	52.7 ± 8.6	0.05 ± 0.02	--[a]
	K	4.0 ± 0.3	0.17 ± 0.00	0.53 ± 0.31	19.8 ± 1.3	0.19 ± 0.03	--[a]
	L	62.4 ± 18.3	0.16 + 0.02	0.05 ± 0.02	26.6 ± 3.3	0.27 ± 0.05	--[a]
Group "0"	M	0.79 ± 0.06	0.0	0.0	35.5 ± 14.7	0.0	0.0
(no with-	K	3.5 ± 0.5	0.0	0.43 ± 0.07	18.0 ± 1.7	0.0	0.0
drawal)	L	37.3 ± 36.3	0.0	0.12 ± 0.06	30.8 ± 0.47	0.0	0.0
Group "10"	M	0.76 ± 0.06	0.0	0.05 ± 1.1	31.9 ± 4.9	0.1 ± 0.2	0.1 ± 0.2
(10 day	K	3.8 ± 0.54	0.0	0.46 ± 0.44	18.7 ± 3.3	0.1 ± 0.2	0.1 ± 0.2
withdraw)	L	29.1 ± 8.3	0.0	0.09 ± 0.03	32.5 ± 5.7	0.1 ± 0.2	0.1 ± 0.2
Group "20"	M	0.74 ± 0.03	0.0	0.0	37.7 ± 9.1	0.0	0.0
(20 day	K	4.1 ± 0.90	0.0	0.45 ± 0.05	20.7 ± 6.7	0.29 ± 0.2	0.29 ± 0.2
withdraw)	L	32.7 ± 8.6	0.0	0.08 ± 0.02	34.6 ± 1.0	0.0	0.0
Control	M	0.84 ± 0.06	0.0	0.0	35.3 ± 4.0	0.0	0.0
(no	K	3.5 ± 0.57	0.0	0.49 ± 0.16	18.0 ± 0.28	0.0	0.0
manure)	L	40.6 ± 11.6	0.0	0.21 ± 0.26	27.7 ± 5.6	0.2 ± 0.23	0.2 ± 0.23

[a] Not done

SWINE FEEDING TRIALS WITH SLUDGES

SECTION A. Performance and Tissue Minerals of Swine Fed Sewage Sludge Diets. J. Beaudouin, R. Shirley, and D. Hammell, 1976

SECTION B. Effect of Feeding Sewage Sludge Diets on Reproduction, Growth, and Tissue Mineral Accumulation. D. Hammell and G. Edds, 1977

SECTION C. Effect of Feeding Digested Sewage Sludge on Long-Term Sow Reproduction Performance. F. White, D. Hammell, . Shepherd, O. Osuna, and G. Edds, 1978; 1979

A. PERFORMANCE AND TISSUE MINERALS OF SWINE FED SEWAGE SLUDGE DIETS

ABSTRACT

Twelve female swine were fed in a 3 x 4 cross-over design metabolism trial corn-soybean grower diets that contained 0, 10, or 20% sewage sludge over 3 19-day periods. The mean values for total digestible nutrients were 79.4, 73.7, and 55.0%; those for metabolizable energy were 3.36, 2.25, and 1.15 Mcal per kg diet; and those for nitrogen retained were 42.8, 44.0, and 25.3%, respectively. Sewage sludge (0, 10, 20%) diets were fed to 31 sows approximately equally divided in the dietary groups during their first 2 pregnancies and to their offspring from weaning to market weight. More live pigs were farrowed and weaned per litter from sows fed 20% sludge diets than from the control group. However, 20 day weaning weights of pigs were lower with sows fed the sludge-containing diets. Offspring of both first and second litters fed growing and finishing diets containing sludge from weaning to market weight had decreased daily weight gains and feed efficiency. There were no increases in the 9 mineral elements (Pb, Cd, Ni, Zn, Cr, Cu, Mn, Fe, and Al) in sow milk or blood. Offspring of sows fed sludge diets showed increases of several elements in selected tissues at weaning and after consuming sludge diets to market weight.

INTRODUCTION

Kienholz *et al.* (1976) found aged cows that grazed pasture for 5 years that was treated with sewage sludge had no marked differences in heavy metal concentration in their tissues. However, steers fed 15% dried sewage sludge in feedlot diets for 94 days had decreased body weight gains and a 10-fold increase in lead and mercury and a 2-fold increase in cadmium and copper in their liver and kidney. Kinzell fed 0 and 30% levels of activated sewage sludge (AcSS) in diets to rats for 3 generations and concluded that performance and tissue heavy metal levels were such that AcSS seemed to have potential as a feed ingredient. The purpose of this study was to determine the effect of sewage sludge in diets fed sows on digestibility of nutrients, reproduction, growth of offspring, and mineral accumulation in tissues.

EXPERIMENTAL PROCEDURE

Metabolism Trial

Twelve gilts averaging 28 kg in body weight were equally divided into 3 groups of 4 and fed corn-soybean grower diets (Table 1) containing either 0, 10, or 20% sewage sludge over 3 19-day periods in a cross-over design metabolism trial. The pigs were randomly selected from several litters of the same age and weight that had not previously been fed sewage sludge diets. The sludge was anaerobically digested in the University of Florida sewage plant, sun-air dried, and replaced corn grain diets on an equal air-dry weight basis. Chromic oxide (0.1% Cr_2O_3) was added as a reference substance and used to calculate total fecal output by the equation:

$$\text{Fecal output} = \frac{\text{Total Cr intake}}{\text{Concentration Cr/g dry matter of feces}}$$

The swine were adapted to the diets for 9 days in pens and then confined to individual metabolism cages where feed intake was recorded and feces and urine collected during days 12-19. Proximate analyses constituents were determined in the sludge, diets, feces, and urine (Table 2) by AOAC (1975) methods.

Reproduction and Weanling Pig Performance

Thirty-three York-Hampshire crossbred gilts that weighed approximately 60 kg each were allotted randomly and equally to corn-soybean gestation-lactation diets (Table 1) that contained either 0, 10, or 20% dried Florida sewage sludge (DFS). During approximately 12 months of feeding the diets, 2 litters were obtained from each of the sows. The sows were fed from troughs in open pens on the ground except just prior to farrowing and during lactation until weaning when they were kept on concrete in farrowing pens and hand-fed. After weaning, the sows were returned to open pens on the ground. Milk from lactating sows and tissues from randomly selected weanling pigs, market weight offspring, and dams from the dietary groups were analyzed for lead, cadmium, nickel, copper, chromium, zinc, manganese, aluminum, and iron by atomic absorption spectrophotometry (Anonymous, 1973). Milk was obtained on the seventh day of the first lactation period from 3 sows randomly selected from each dietary group. It was dried on a steam bath, ashed at 550^{o} overnight, ash dissolved in 0.1% HCl, and the above elements analyzed.

Six weanling pigs (3 male, 3 female) of each dietary group of both first and second litters were killed by jugular incision and blood obtained in heparinized tubes. Approximately 10 minutes later, liver, kidney, spleen, and muscle (ham) tissues were secured with a stainless steel blade and frozen immediately at $-10^{o}C$. Samples were partially oxidized with nitric acid followed by dry ashing at $480^{o}C$. The ash was dissolved in aqueous nitric acid and analyzed for the selected elements. Eighteen of the brood sows (6 from each dietary group) were randomly selected and slaughtered after weaning their second litters. The liver, kidney, spleen, muscle, and blood were collected and handled as above prior to analyses of the 9 elements.

Growing and Finishing Trial

Seventy-two weanling pigs of each of the first and second litters were randomly selected and divided equally into 3 groups and fed either 0, 10, or 20% DFS-containing growing and finishing diets (Table 1) to market weight (approximately 80 to 90 kg). In this trial, pigs were not changed from the dietary sewage sludge levels of their dams. Pigs of each set of litters were allotted randomly to 18 pens according to weight, sex, and litter number, with 2 barrows and 2 gilts in each pen (non-littermates). Individual weights and pen feed consumption data were obtained every 2 weeks. At market weight, 18 animals (9 barrows, 9 gilts), 1 from each pen, were randomly selected, slaughtered, and blood, liver, kidney, spleen, and muscle collected and handled as above for analyses of selected elements (Anonymous, 1973).

Statistical Analysis

In the metabolism trial, significance of variance in values due to treatments were analyzed using the least significant difference (LSD) method of Steel and Torrie (1960). The data in all other treatments were analyzed for significance of variances by the SAS computer method (Barr *et al.*, 1976). Duplicate analyses were made for each element in the various tissues.

RESULTS AND DISCUSSION

Digestibility Trial

The digestibility of crude protein, crude fiber, ether extract, ash, and nitrogen-free extract as well as total digestible nutrients (TDN), metabolizable energy (ME) and nitrogen balance data are presented in Table 3. All proximate analyses nutrient values except ether extract were decreased ($P < 0.05$) in digestibility when 20% sludge was in the diet; as well as TND, ME, and nitrogen retained. It is not apparent why more ether extractable material was present in the feces than in the diet which ranged from 0.3 to 1.1% (Table 2). It must have been of metabolic origin.

Reproductive and Weanling Pig Performance

The reproductive performance of sows fed 10 and 20% sewage sludge diets was not adversely affected during their first and second pregnancies compared to the controls. There were apparent linear decreasing weights of pigs from dams fed 10 and 20% sewage sludge diets (Table 4). Approximately 1 to 2 more live pigs were farrowed on the average and slightly more (0.25 to 0.45) pigs were weaned per litter with diets than contained 20% sludge. However, the 21 day old weaning weights were depressed by dietary sludge. Dams fed the 20% sludge diets had weaning weights of offspring that averaged 0.53 and 1.45 kg less (not significant) than controls for the first and second litters, respectively. The slightly slower gaining pigs appeared to be normal in vigor and overall health.

Growing-Finishing Swine Performance

In Table 5 are presented performance data on first and second litter offpsirng fed growing-finishing diets that contained 0, 10, and 20% DFS. The swine from the second litters fed the sludge-containing diets had less ($P < 0.05$) daily weight gains and a greater ($P < 0.05$) feed gain ratio than the controls. The first litter swine fed diets with sludge increased their feed intake over the controls and made similar weight gains.

They had greater ($P < 0.05$) feed:gain ratio. The failure of the second litter swine to consume more of the sludge-containing diets than the controls as was done by the corresponding first litter group may have been due to the stress of summer temperature as they were born in June and the first litters were born in January. Similar weight gain depression to that observed with the second litter groups was reported by Kienholz *et al.* (1976) upon feeding 5 or 15% dried sewage sludge diets to feedlot steers.

Mineral Accumulation in Milk and Tissue

Data obtained on mineral accumulation in milk and tissues of sows slaughtered after weaning their second litters are presented in Table 6. The sludge diets resulted in no increase in concentration of selected elements in milk or blood of the sows. However, sows fed the 10 to 20% sludge-containing diets had an increase in cadmium in the kidney and muscle (10% sludge only); nickel in the liver, kidney (20% sludge only), spleen and muscle; copper in the liver; chromium in the liver, kidney, spleen, and muscle; zinc in the kidney (10% sludge only); manganese in the liver, and aluminum in the liver, kidney, spleen, and muscle. Similar concentrations of several elements in swine liver to those found in the present study have been reported (Liebholz *et al.*, 1962; Hedges and Kornegay, 1973; Gipps *et al.*, 1974; and Skutches *et al.*, 1974). Apparently there is no corresponding diet versus tissue data of swine for cadmium, nickel, chromium, and aluminum. Kienholz *et al.* (1976) fed diets that contained 15% sewage sludge for 94 days to feedlot steers and found liver and kidney had a 10-fold increase in lead and mercury, and a 2-fold increase in cadmium and copper, but no histological or pathological changes in tissues.

In Table 7 are presented concentrations of various elements in selected tissues of weanling offspring from the first and second litters of sows fed diets that contained 0, 10, and 20% DFS. The liver of those fed sludge-containing diets had more ($P < 0.05$) lead, cadmium, copper, and iron in the first litters, but not in the second ones. Aluminum was increased in those fed the 20% sludge level in the second sludge diet but not in the first litters. Nickel was increased with the 10% sludge diet but not at the 20% level with the first litters. The sludge diets had no effect on chromium, zinc, and manganese in the liver. In the kidney, the concentration of lead, cadmium, nickel, copper, chromium, zinc, aluminum, and iron were increased in weanling pigs of the first litters of dams fed 20% sludge diets; and the same was true of cadmium, nickel, and chromium with the corresponding first litters. Manganese in the kidney was not affected by the dietary sludge. In the spleen, nickel, copper, and manganese were greater with weanling pigs of the first litters of dams fed diets that contained 20% sewage sludge. Manganese was also greater at the 10% level in this treatment group. Chromium was higher in the spleen of corresponding pigs of the second litters of dams fed 20% sludge diets. Concentrations of lead, cadmium, zinc, aluminum, and iron in the spleen was not effected by the sludge-containing diets. In the skeletal muscle, cadmium, nickel, chromium, zinc, and manganese were greater in the first litters of dams fed 20% sludge diets, but all except cadmium were not effected in the second litters; cadmium was less. The sludge-containing diets had no effect on lead, cadmium, copper, aluminum, and iron in muscle.

In the blood, lead and cadmium were more concentrated in offspring of second litters of dams fed 20% sludge diets; but not in those of the first litters. Chromium was greater in corresponding first litter pigs of dams fed 20% sludge diets; and iron was less in the blood of the second litter pigs. The dietary treatments had no effect on copper, zinc, manganese, and aluminum concentration in the blood.

Concentrations of selected elements in various tissues at time of slaughter of the growing-finishing offspring fed diets that contained 0, 10, or 20% sewage sludge are presented in Table 8. The sludge-containing diets fed the first litter offspring had no effect on concentrations of any of the 9 elements in the liver. However, in the second litters, cadmium of the 20% sludge groups and zinc of both sludge groups were greater than the controls. The kidney of the first litters was not effected by the sludge diets. But the kidneys of the second litter swine had more cadmium with both sludge levels. The sludge diets had no influence on mineral deposition in the spleen of either the first or the second litters. Lead was greater in the muscle of the second litter pigs fed the 20% sludge-containing diet, while zinc was lower in muscle of second litter offspring that consumed 10 ro 20% sludge diets. None of the minerals were elevated in blood due to the dietary sludge.

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TABLE 1. BASAL FIETS FED DURING METABOLISM TRIAL, GESTATION-LACTATION, GROWING AND FINISHING PERIODS[a]

	Internat'l Ref. No.[b]	Gestation and lactation	Starting	Grower and metabolism	Finishing
Corn, grain, yellow, grond (9.0%)	4-02-931	70.45	61.45	78.25	83.25
Oats, grain, grnd (12.7%)	4-03-315	10.0	-	0	0
Sugarcane, molasses, 79.5° brix	4-04-696	-	5.0	-	-
Soybean seed, dehulled, solv extract	5-04-612	16.0	27.5	18.75	13.75
Animal fat	4-00-409	-	3.0	-	-
Phosphate, defluorinated[c]	6-01-780	2.5	2.0	1.50	1.50
Salt, iodized	6-04-151	.5	.5	.50	.50
Trace mineral premix[d]		.05	.05	.10	.10
Vitamin premix[e]		.5	.5	.50	.50
Limestone, grnd	6-01-069	0	-	.40	.40

[a] Corn was replaced on air-dry weight basis by sewage sludge in 10 and 20% sludge diets fed swine.

[b] Atlas of Nutritional Data on United States and Canadian Feeds, 1971. Nat. Acad. of Sci., Wash., DC.

[c] Courtesy of Occidental Petroleum Co., White Springs, FL.

[d] Provided the following elements in mg per kg diet: zinc (ZnO), 200; iron ($FeSO_4$), 100; manganese ($MnSO_4$), 55; copper (Cu_2O), 11; iodine $Ca(IO_3)_2$, 1.5; and cobalt ($CoCO_3$), 1.0. Courtesy of Calcium Carbonate Company, Quincy, IL.

[e] Provided the following vitamins per kg diet: vitamin A, 11,025 IU; vitamin D, 5,513 IU; vitamin E, 22 IU; riboflavin, 11 mg; pantothenic acid, 27 mg; niacin, 55 mg; choline chloride, 1,102 mg; vitamin B_{12}, 49 mcg. Courtesy of Charles Pfizer and Company, Terre Haute, IN.

TABLE 2. PROXIMATE ANALYSES AND SELECTED MINERAL COMPOSITION OF SEWAGE SLUDGE AND DIETS

	Air dry matter %	Proximate analyses[a] Cr. protein %	Cr. fiber %	Ether extract %	NFE[b] %	Ash %
Sludge[c]	91.9 (66-98)	25.9 (22-37)	4.3 (.7-15)	.46 (.01-1.1)	40.6 (9-47)	23.8 (27-57)
Metabolism trial:						
Control diets (grower)						
Period 1	85.9	17.5	6.0	1.1	69.5	5.9
Period 2	84.5	21.9	4.6	.4	64.7	8.4
Period 3	87.2	19.5	12.7	.7	55.5	11.7
Sludge diets, 10%						
Period 1	84.4	19.4	6.4	.6	66.2	7.4
Period 2	89.1	18.4	6.9	.4	66.3	8.1
Period 3	85.2	19.7	3.5	.4	64.9	11.5
Sludge diets, 20%						
Period 1	83.9	25.0	4.5	.6	63.2	6.8
Period 2	84.1	20.2	6.0	.3	65.1	8.5
Period 3	82.4	21.4	5.7	.6	61.9	10.5
Gestation-lactation diets						
Control	84.7	20.6	5.7	.8	66.3	6.7
Sludge, 10%	85.9	20.1	5.8	.4	65.1	8.3
Sludge, 20%	84.9	20.2	7.3	.5	61.9	11.2

Minerals, mg/kg dry matter	Pb	Cd	Ni	Zn	Cu	Cr	Mn	Fe	Al
Sludge	463	13	32	1190	512	213	274	10670	4436
Diets:									
Control	2	.3	4	189	12	2	75	344	419
Sludge, 10%	31	1.3	5	265	42	16	90	1011	688
Sludge, 20%	66	2.2	8	381	80	32	118	1963	1045

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[a] Percentage on dry matter basis

[b] Nitrogen-free extract

[c] Mean and range of 22 samples

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TABLE 3. DIGESTIBILITY OF DRY MATTER, ORGANIC MATTER, CRUDE PROTEIN, ETHER EXTRACT, CRUDE FIBER, NITROGEN-FREE EXTRACT (NFE), TOTAL DIGESTIBLE NUTRIENTS (TDN), METABOLIZABLE ENERGY (ME), AND NITROGEN RETAINED IN SEWAGE SLUDGE DIETS FED SWINE

	Control	Sludge, 10%	Sludge, 20%
Number of swine	12	12	12
Daily intake, kg, dm	1.59	1.57	1.74
Digestibility (%)			
Dry matter	76.4^{b}	68.3^{b}	49.8
Organic matter	79.7^{b}	72.5^{b}	56.5
Crude protein[a]	77.8^{b}	68.5^{bc}	58.2
Ether extract[a]	-63.6^{b}	-42.9^{c}	-112.5
Crude fiber[a]	69.7	70.3	62.9
NFE	82.8^{b}	78.2^{b}	61.7
TDN (%)[a]	64.7^{b}	62.3^{b}	53.6
ME, Mcal/kg[a]	3.74^{b}	2.74^{bc}	1.88
Nitrogen retained (%)[a]	50.9^{b}	51.4^{b}	35.6

[a] Dry matter basis

[b,c,d] Values on lines that vary significantly have different superscript letters ($P < 0.05$)

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TABLE 4. REPRODUCTIVE PERFORMANCE OF SOWS DURING FIRST AND SECOND LITTERS ON DIETS CONTAINING 0, 10, and 20 PERCENT SEWAGE SLUDGE

Item	First litter			Second litter		
	0%	10%	20%	0%	10%	20%
Number mated	11	11	11	9	10	10
Number farrowed	10	10	10	7	9	8
Breeding weight, kg	152.2	148.2	145.4	164.5^a	167.8^a	155.1^b
Gestation period, days	113.4	115.2	114.4	114.6	114.6	114.5
Gestation weight gain, kg	42.9^a	40.3^a	30.1^b	47.4^a	41.8^a	31.9^b
Total pigs farrowed per litter	9.9	9.3	11.2	9.0	9.2	10.4
Live pigs farrowed per litter	7.4	7.6	9.5	8.3	8.7	9.4
Birth weight of live pigs, kg	1.19	1.24	1.09	1.44	1.24	1.39
Weaning weight/pig (21-day), kg	5.02	4.98	4.49	6.28	5.36	4.83
Pigs weaned/litter	6.55	6.80	7.00	8.00	7.11	8.25

^{a,b} Means having same superscript letter do not differ significantly ($P < 0.05$)

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TABLE 5. PERFORMANCE OF FIRST AND SECOND LITTER OFFSPRING FED GROWING AND FINISHING DIETS THAT CONTAINED 0, 10, AND 20 PERCENT SEWAGE SLUDGE

Final weight, kg	94.50	92.90	91.40	95.41	86.91	81.63
Initial weight, kg	8.91	8.95	9.22	10.36	8.36	8.45
Weight gain, kg	85.59	83.95	82.10	85.05	78.55	73.10
Days on test	133	133	133	128	128	128
Average daily gain, kg	.64	.63	.62	.66^{a}	.60^{b}	.56^{b}
Average daily feed, kg	1.94^{a}	2.10^{ab}	2.30^{b}	2.03	1.99	1.86
Feed:gain ration	3.06^{a}	3.34^{b}	3.69^{b}	3.05^{a}	3.36^{b}	3.39^{b}

[a,b] Means in same row that vary significantl have different superscript letters ($P < 0.05$)

TABLE 6. EFFECT OF SEWAGE SLUDGE IN DIETS FED SOWS ON CONCENTRATION OF MINERALS IN SELECTED TISSUES[a,b]

Diet	Pb	Cd	Ni	Cu	Cr	Zn	Mn	Al	Fe
Milk, mg/liter									
Control	1.0	.06	.1	1.0	<.01	9	.2	25	2
Sludge, 10%	1.3	.06	.1	1.1	<.01	9	.2	26	2
Sludge, 20%	1.0	.05	.1	1.2	<.01	10	.1	24	1
Liver, mg/kg dry wt									
Control	<.01	2	3	39	1	231	7	259	1217
Sludge, 10%	2	5	14	64	11	243	11	763	1133
Sludge, 20%	<.01	4	23	51	12	209	9	873	1126
Kidney, mg/kg dry wt									
Control	<.01	4	5	26	1	122	6	397	875
Sludge, 10%	<.01	17	10	54	8	123	5	1952	392
Sludge, 20%	<.01	24	20	44	15	147	6	1069	451
Spleen, mg/kg dry wt									
Control	<.01	.4	8	10	<.01	128	<.05	483	3030
Sludge, 10%	<.01	2	17	18	15	127	<.05	1123	4736
Sludge, 20%	<.01	.03	19	17	16	123	<.05	1062	5104
Muscle, mg/kg dry wt									
Control	<.01	1	4	12	.01	113	2	485	689
Sludge, 10%	<.01	2	11	15	12	111	.6	836	304
Sludge, 20%	<.01	<.03	21	10	14	90	<.05	1006	125
Blood, mg/liter									
Control	2	1	9	13	7	33	.5	384	2051
Sludge, 10%	<.01	2	8	12	<.01	28	<.05	139	2289
Sludge, 20%	<.01	1	9	7	.1	29	<.05	374	2181

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[a] Milk from 3 sows/treatment during seventh day of the first lactation period.

[b] Tissues from 6 sows/treatment slaughtered after second pregnancy and lactation period.

[c] The <symbol denotes limit of measurement by procedure

[d,e,f] Means that have same superscript letter do not differ significantly ($P < 0.05$)

TABLE 7. MINERALS IN WEANLING PIG TISSUES OF FIRST AND SECOND LITTERS

Sludge in diet %	Lead		Cadmium		Nickel		Copper		Chromium	
	1st^{a}	2nd^{a}	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Liver, mg/kg, dry basis										
0	<.01^{b}	7.8	<.03	.9	<.01	.9^{b}	108^{b}	11	<.01	<.01
10	<.01^{b}	6.2	<.03	1.1	<.01	2.9^{c}	101^{b}	13	<.01	.62
20	6.51^{c}	5.1	1.06	1.5	<.01	1.7^{b}	85^{c}	12	.10	.86
Kidney, mg/kg dry basis										
0	<.01^{b}	8.0	<.03^{b}	3.5^{b}	< .1^{b}	.7^{b}	32^{b}	20	<.01	<.01^{b}
10	<.01^{b}	9.9	<.03^{b}	6.2^{c}	< .1^{b}	1.9^{c}	37^{b}	19	.16	.22^{b}
20	11^{c}	8.0	4.57^{c}	6.8^{c}	24^{c}	2.1^{c}	48^{c}	21	.71	2.02^{c}
Spleen, mg/kg, dry basis										
0	<.01	13.6	.33	1.3	.1^{b}	2.2	18^{b}	8.2	.5	.4^{b}
10	<.01	11.4	.65	1.3	7.8^{c}	4.7	15^{b}	5.4	.8	.01^{b}
20	<.01	7.9	.03	1.9	14.5^{d}	3.9	30^{c}	6.7	.1	2.1^{c}
Muscle, mg/kg dry basis										
0	<.01	6.0	.30^{b}	.8	< .1^{b}	.9	15	4.0	<.01^{b}	.21^{b}
10	<.01	5.4	.30^{b}	.6	< .1^{b}	1.9	9	2.5	<.01^{b}	.62^{b}
20	<.01	4.4	2.22^{c}	1.1	20.0^{c}	1.5	17	4.2	.62^{c}	<.01^{c}
Blood, mg/liter										
0	7.2	10.8^{b}	1.12	<.03^{b}	5.41^{b}	3.2	9.5	14.3	<.01^{b}	1.9^{b}
10	9.9	11.3^{b}	1.12	<.03^{b}	4.43^{b}	2.5	9.4	11.1	<.01^{b}	1.1^{b}
20	7.6	5.1^{c}	.47	.94^{c}	<.01^{c}	2.6	10.0	8.1	1.03	<.01^{c}

TABLE 7. Continued

Sludge in diet %	Zinc		Manganese		Aluminum		Iron	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Liver, mg/kg, dry basis								
0	336	172	8.2	6.9	1239	441^{b}	274^{b}	476
10	380	160	10.2	7.5	1563	412^{b}	327^{b}	438
20	408	139	10.6	7.8	1336	711^{c}	609^{c}	525
Kidney, mg/kg, dry basis								
0	115^{b}	122	2.7	6.6	1555^{b}	561^{b}	256^{b}	224
10	103^{b}	100	6.1	4.4	1691^{b}	351^{b}	263^{b}	205
20	313^{c}	108	9.2	5.8	2207^{c}	530^{b}	475^{c}	257
Spleen, mg/kg, dry basis								
0	114	132	.05^{b}	2.0	1627	626	566	918
10	95	115	2.62^{c}	1.4	1842	458	616	1029
20	128	119	2.69^{c}	1.8	1896	851	850	1003
Muscle, mg/kg, dry basis								
0	59^{b}	111	$<$.05^{b}	.35^{b}	1235	368	117	52
10	66^{b}	62	.63^{b}	$<$.05^{c}	1474	264	128	52
20	148^{c}	94	4.28^{c}	.30^{b}	1529	282	345	53
Blood, mg/liter								
0	25.7	21	$<$.05	$<$.05	652	440	1901	2172
10	26.8	26	$<$.05	$<$.05	584	342	1881	2186
20	29.7	20	$<$.05	$<$.05	607	344	1956	1800

[a] 1st and 2nd refers to first and second litters; each value is the mean of 6 pigs (3 male, 3 female) per diet; sex had no effect

[b,c,d] Means that have different superscript letters differ significantly with various treatments ($P < 0.05$)

TABLE 8. MINERALS IN TISSUES OF GROWING-FINISHING SWINE FROM FIRST AND SECOND LITTERS FED DIETS CONTAINING SEWAGE SLUDGE (6 PER TREATMENT)

Sludge in diet	Lead		Cadmium		Nickel		Copper		Chromium	
%	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Liver, mg/kg, dry basis										
0	7.3	1.72	1.00	.33	5.2	<.1	44	14	.63	<.01
10	8.6	<.01	1.19	.84	3.2	.7	31	17	.85	2.77
20	7.2	<.01	.56	3.18	3.4	6.6	19	17	.16	8.05
Kidney, mg/kg dry basis										
0	6.8	<.01	.76	1.40	4.2	<.1	19	22	<.01	<.01
10	11.4	<.01	2.50	8.60	7.7	.9	32	27	1.1	3.04
20	12.7	<.01	1.28	12.5	4.7	<.1	25	27	.22	2.18
Spleen, mg/kg, dry basis										
0	12.1	1.84	1.03	.64	4.9	1.3	13	10	<.01	<.01
10	14.4	<.01	1.73	<.03	5.5	1.0	16	11	<.01	1.84
20	10.1	<.01	.67	<.03	4.6	4.7	15	6	.22	7.06
Muscle, mg/kg, dry basis										
0	3.8	2.14	.79	.75	2.3	<.1	9	9	.62	<.01
10	4.2	<.01	.72	<.01	2.5	1.2	6	7	.43	3.05
20	7.0	<.01	.64	<.01	3.2	3.1	10	8	1.15	2.18
Blood, mg/liter										
0	5.3	11.2	.07	1.80	2.5	.7	11	9	1.01	<.01
10	7.1	9.7	.15	1.29	1.8	.5	11	11	.87	<.01
20	4.6	10.6	.16	4.47	1.2	<.1	9	13	.30	<.01

TABLE 8. Continued

Sludge in diet, %	Zinc		Manganese		Aluminum		Iron	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
				Liver, mg/kg, dry basis				
0	172	109^b	6.2	9.3	441	687	519	615
10	143	136^c	5.9	6.8	472	587	614	507
20	168	176^d	5.1	8.3	401	853	558	546
				Kidney, mg/kg, dry basis				
0	130	95	4.3	15.7	563	668	296	269
10	177	96	7.0	4.4	781	582	585	242
20	197	139	5.8	5.8	552	612	663	285
				Spleen, mg/kg, dry basis				
0	157	104	3.3	1.6	1000	688	1081	758
10	145	118	3.7	.9	557	596	2507	764
20	131	106	3.7	.6	446	1073	1772	839
				Muscle, mg/kg, dry basis				
0	112	99^b	2.4	.51	330	486	227	112
10	85	76^c	2.1	<.05	393	545	239	88
20	137	69^c	3.7	<.05	517	665	392	87
				Blood, mg/liter				
0	17	24	.6	<.05	259	479	1884	221
10	17	26	<.1	<.05	216	348	1676	229
20	18	24	<.1	<.05	259	418	1491	223

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[a] First and second litters

[b,c,d] Values in same column with different superscripts are different ($P < 0.05$)

B. EFFECT OF FEEDING SEWAGE SLUDGE DIETS TO SWINE ON REPRODUCTION, GROWTH, AND TISSUE MINERAL ACCUMULATION

ABSTRACT

A total of 39 gilts initially weighing 77 kg body weight were allotted on the basis of weight and ancestry. Animals were allowed 1.81 kg daily of a corn-soy diet containing 0, 10, or 20% municipal sewage sludge from the University of Florida waste treatment plant (DFS) for 2 gestation periods and were allowed *ad libitum* consumption of the same diet during lactation. Gilts fed 0, 10, and 20% sludge diets weaned 7.91, 6.89, and 7.22 pigs/litter, respectively, at 21 days of age. Corresponding number of pigs weaned/litter at 21 days of age from second litter sows indicate sows fed 0% added sludge weaned more pigs (P 0.10) than those fed sewage sludge diets (8.00 versus 6.27, 6.30). After weaning, gilt offpsring were continued on the same sludge dietary treatment as their dam. Average daily gain, average daily feed, and feed required per unit of gain for offspring (18 per treatment, 6 replications) during the growing-finishing period were: 0.61, 0.60, 0.56; 1.88^a, 2.10^b, 2.08^b; 3.06^a, 3.59^b, 3.70^b, for those fed diets containing 0, 10, and 20% sludge respectively. Corresponding performance values for gilt offspring (24 per treatment, 6 replications) fed sludge from Chicago which was substituted on a kg per kg basis for University of Florida sludge were: 0.65^a, 0.60^a, 0.49^b; 1.84^a, 1.83^a, 2.09^b; 2.84^a, 3.08^a, 4.26^b. Following the termination of each growing-finishing experiment, 6 pigs per treatment (18) were slaughtered and concentration of Hg, Cu, Fe, Zn, Cr, Ni, Cd, Co, and Pb were determined in liver, muscle, and kidney tissue. Of greatest concern was the accumulation of Cd in the kidney of offspring fed diets containing dried Chicago sludge. Means with like superscripts do not differ significantly ($P < 0.05$).

TABLE 9. SLUDGE AND RATION MINERAL ANALYSIS

Mineral ppm	Dried U of F Sludge (DFS)	Grower-Finisher Diet Control	10% Sludge	20% Sludge
Hg	81.7	0.02	3.86	8.17
Cu	555.9	12.86	41.3	81.36
Fe	4,100.0	285.5	536.25	908.75
Zn	1,216.7	204.38	216.25	346.00
Cr	217.6	2.08	11.34	25.02
Ni	24.9	1.04	2.50	5.00
Cd	9.1	.18	.938	1.39
Co	7.9	1.88	1.67	2.89
Pb	416.8	1.72	19.22	45.78

Mineral ppm DM	Dried U of F Sludge (DFS)	Control	10% Sludge	20% Sludge
Hg	81.7	-	-	-
Cu	555.9	8.65	46.43	102.10
Fe	9,366.6	269.00	3,750.00	984.00
Zn	1,216.7	123.22	568.33	303.83
Cr	217.6	1.46	18.96	37.52
Ni	24.9	1.04	3.00	7.61
Cd	9.1	.365	3.81	2.21
Co	7.9	1.67	4.17	4.17
Pb	416.8	0.83	19.58	76.25

TABLE 10. SLUDGE AND RATION MINERAL ANALYSIS

Mineral ppm	Dried Chicago Sludge	Grower-Finisher Diet Control	Grower-Finisher Diet 10% Sludge	Grower-Finisher Diet 20% Sludge
Hg	1.79	0.02	1.85	5.05
Cu	1,330	18.9	107.1	200.94
Fe	49,862	236.6	2,616	5,244.4
Zn	3,230	236.7	349.1	557.66
Cr	3,350	2.43	179.85	324.12
Ni	380	2.72	31.77	57.12
Cd	218	0.10	9.26	17.15
Co	17.91	2.0	2.74	4.31
Pb	715	1.0	38.76	86.1

Detection Limits - PPM Dry Matter

Aluminum, 0.5; Cadmium, 0.025; Cobalt, 0.10; Chromium, 0.10; Copper, 0.05; Iron, 0.05; Lead, 0.10; Magnesium, 0.01; Manganese, 0.05; Mercury, 0.002; Nickel, 0.10; Selenium, 0.10; Zinc, 0.01

TABLE 11. REPRODUCTIVE PERFORMANCE OF GILTS

	Sewage Sludge (%)		
	0	10	20
Gilts mated	11	11	11
Gilts farrowed	11	9	9
Breeding wt, kg	120.6	119.4	115.4
Farrowing wt, kg	169.4	160.3	151.0
Total pigs farrowed/litter	10.54	9.44	9.67
Live pigs farrowed/litter	9.18	8.11	9.00
Birth wt/pig (live), kg	1.41	1.49	1.34
Lactation wt loss, kg	25.4	12.9	19.2
21-day wean wt/pig, kg	5.39	5.24	4.72
Pigs weaned/litter	7.91	6.89	7.22

TABLE 12. REPRODUCTIVE PERFORMANCE OF SOWS

	Sewage Sludge (%)		
	0	10	20
Gilts mated	11	11	11
Gilts farrowed	10	11	10
Breeding wt, kg	144.1$_a$	147.4$_a$	131.7$_b$
Farrowing wt, kg	192.0^a	190.0^a	162.3^b
Total pigs farrowed/litter	10.6	10.0	9.1
Live pigs farrowed/litter	9.90	9.63	8.30
Birth wt/pig (live), kg	1.51$_a$	1.58$_a$	1.47$_b$
Lactation wt loss, kg	34.4^a	33.5^a	16.5^b
21-day wean wt/pig,kg	5.11	4.86$_d$	5.24$_d$
Pigs weaned/litter	8.00^c	6.27^d	6.30^d

^{a,b,c,d} Means bearing like superscripts do not differ significantly ($P < 0.05$ and $P < 0.10$, respectively)

TABLE 13. GILT OFFSPRING FED CHICAGO SLUDGE

	Sewage Sludge (%)		
	0	10	20
Average daily gain, kg	0.65^{a}	0.60^{a}	0.49^{b}
Average daily feed, kg	1.84^{a}	1.83^{a}	2.09^{b}
Feed/Gain	2.84^{a}	3.08^{a}	4.26^{b}

[a,b] Means in same row bearing like superscripts do not differ significantly ($P < 0.05$)

TABLE 14. GILT OFFSPRING FED GAINESVILLE SLUDGE

	Sewage Sludge (%)		
	0	10	20
Average daily gain, kg	0.61	0.60	0.56
Average daily feed, kg	1.88^{a}	2.10^{b}	2.08^{b}
Feed/Gain	3.16^{a}	3.59^{b}	3.70^{b}

[a,b] Means in same row bearing like superscripts do not differ significantly ($P < 0.05$)

C. EFFECT OF FEEDING DIGESTED SEWAGE SLUDGE ON LONG-TERM SOW REPRODUCTIVE PERFORMANCE

ABSTRACT

Gilts selected from 2 generations in succession were fed diets containing 0, 10, or 20% dried sewage sludge (DFS) in a basal corn-soybean mean formulation. In order to assess long-term effects, continuity in the experiment was maintained by feeding second generation females the same dietary regimen fed to their dams.

Data collected indicated that feeding sewage sludge to female swine over an extended period adversely affected many criteria used in evaluating reproductive performance. Breeding, farrowing, and rebreeding weights were reduced. Lactation and gestation weight changes were lower and fewer pigs were farrowed in sow groups receiving 10 and 20% sewage sludge in diets. First and second generation sows fed diets containing 10 and 20% sewage sludge weaned lighter pigs at 21 days when compared with sows fed the basal diet. In each parity, pigs farrowed by sows fed sewage sludge in diets displayed depressed average daily gain. A comparison of the data from both generations receiving diets containing sewage sludge indicate that reproductive performance was more diminished in second generation sows than in first.

INTRODUCTION

Beaudouin *et al.* (1980) fed diets containing 0, 10, and 20% DFS to female swine during their first 2 pregnancies, and to their offspring from weaning to market weight. Their data show that all proximate analysis nutrients except ether extract were decreased in digestibility when 20% DFS was in the diet. Total digestible nutrients, metabolizable energy, and nitrogen retained were also decreased by the diet containing 20% DFS. Their data also indicate that breeding and gestation weights in sows and birth and weaning weights in progeny were lower in groups receiving 20% DFS in diets. Conversely, numbers of total and live pigs farrowed and pigs weaned per litter were higher in sow groups fed the diet containing 20% DFS, but means were not significant. Feeding diets containing 10 and 20% DFS to growing-finishing pigs reduced daily gains when compared with pigs fed the control diet.

The objectives of this experiment were:

1. To evaluate dried University of Florida sewage sludge (DFS) as a source of nutrients for swine.

2. To observe the reproductive performance of female swine fed diets containing DFS throughout 5 parities over 2 sow generations in succession.

The first objective yielded information concerning the feeding value of DFS while the second objective revealed possible long-term effects of sludge-containing diets on swine reproduction.

METHODS AND MATERIALS

Sixty-six gilts (33 from each of 2 successive generations) of predominantly Yorkshire X Hampshire ancestry, were allotted randomly to receive an experimental diet containing either 0, 10, or 20% DFS. Second generation gilts were selected from the second parity of first generation sows (Figure 1). Data reporting reproductive performance of first generation sows and postpartum performance of their progeny were collected during 3 successive farrowing. Data from second generation sows and their progeny were collected during 2 successive farrowings. Second generation sows received the same experimental diet fed to their dams.

Following breeding, females were moved to outside dirt lots that were devoid of vegetation. During prebreeding, breeding, and gestation, animals were group fed their assigned diet at the level of 1.81 kg/day. During lactation, sows were allowed 5.44 kg/day consumption of the same diet. Diets were formulated from corn-soybean meal (Table 15). Corn was replaced by DFS on a pound to pound basis to achieve the desired treatment ratios.

Sows were routinely dewormed with Dichlorovos 14 days postpartum and were washed, sprayed for external parasites, weighed, and moved into a central farrowing barn 107 days postcoitus. Data evaluating individual sow and progeny performance to sow dietary treatment received were collected at parturition and weaning (21 days postpartum). Sows were rebred on the first estrus following weaning. Differences in treatment and parity means in both sow generations were subjected to an analysis of variance, completely random design (Steel and Torrie, 1960) performed by a computerized general linear model of the statistical analysis system (SAS). Differences among treatment and parity means were calculated using the student's "t" test. DFS was obtained from the University of Florida, Gainesville sewage treatment plant and is the product of aerobic secondary treatment of predominantly human sewage exposed to trickling filtration, contact stabilization, and 3-stage digestion followed by air drying over sand beds. The proximate analysis of 22 samples of DFS from the University of Florida sewage treatment plant were reported by Beaudouin *et al.* (1980) (Table 16).

RESULTS AND DISCUSSION

Breeding and Farrowing Performance

Breeding and farrowing data and gestation intervals of sows fed diets containing 0, 10, and 20% DFS are presented in Table 17. The number of sows bred at each parity was identical in both generations, although the farrowing percentage of second generation sows in parities 1 and 2 was lower than that of sows in the first generation. Sows in both generations fed diets containing DFS farrowed fewer litters than those fed the basal diet. Following the weaning of litters, first generation sows displayed a higher rebreeding percentage at first estrus than second generation sows. In both first and second generations, the percentage of sows successfully rebred was lower in groups fed diets containing DFS. Gestation length was not affected by dietary treatment and did not differ with parity or sow generation compared.

Sow Weight Response

The effects of adding 0, 10, and 20% DFS to diets fed first and second generation sows on weight changes measured at successive intervals of breeding, farrowing, rebreeding, lactation, and gestation are presented in Table 18.

Breeding weights in first generation sows were reduced by additions of 10 and 20% DFS to diets. Breeding weights in second generation sows were also reduced over parities 1 and 2 when DFS was added to the basal diet. In both first and second generation sows, breeding weights increased between each ensuing parity but were greatest between parities 1 and 2. This observation was attributed to the compensatory weight gain that accompanies advancing age in sows.

Farrowing weights were decreased when 10 and 20% DFS were added to the basal diet in both sow generations but both displayed weight gains between parities 1 and 2. This observation may also be attributed to compensatory weight gain although sows in the first generation exhibited a net weight reduction between parities 2 and 3.

Rebreeding weights were affected by dietary treatment received. Sows fed diets containing 10 and 20% DFS in both generations were consistently lighter at rebreeding between parities than those fed basal diets. As observed with breeding and farrowing weights, means of rebreeding weight increased with ensuing parity in both sow generations.

An inverse relationship was measured between lactation weight change and percent DFS in diets fed first generation sows. In each of 3 successive parities, lactation weight change was generally less in groups receiving diets containing 10 and 20% DFS. In parity 1 of second generation sows, lactation weight change among treatments was similar. However, in parity 2, sows receiving 10 and 20% DFS in diets lost less weight during lactation than sows receiving 0% DFS. Lactation weight change increased between parities 1 and 2, then decreased between parities 2 and 3 in first generation sows. Second generation sows also displayed an increase in lactation weight change between parities 1 and 2.

The addition of 10 and 20% DFS to the sow diet in both first and second generation sows caused a reduction in gestation weights within each parity. Gestation weight gains were inverse to breeding, farrowing, and rebreeding weight gains when means among successive parities were compared. The heaviest gestation weight gains in both first and second generation sows were measured during the first parity and were interpreted as compensatory with age.

Effects of DFS on Progeny Mortality

Table 19 presents data measuring mortality of pigs farrowed by first and second generation sows receiving 0, 10, or 20% DFS in diets.

The total number of pigs farrowed by first generation sows fed diets containing 0, 10, or 20% DFS was similar. However, sows fed the diet containing 20% DFS averaged 1 pig fewer over the 3 parities. Second generation sows fed the diet containing 10% DFS farrowed fewer pigs in parities 1 and 2 than sows receiving the 0 or 20% diets. Litter size remained constant between parities 1 and 2 then declined slightly between parities 2 and 3 in first generation sows. Conversely, litter size was increased appreciably between parities 1 and 2 in second generation sows.

Live pigs farrowed were not greatly affected by dietary treatment fed first and second generation sows. The number of live pigs farrowed by first generation sows increased slightly between parities 1 and 2 then declined between parities 2 and 3. Second generation sows farrowed a much larger number of pigs in parity 2 than in parity 1.

The number of stillborn pigs was not influenced by treatment diets fed to first generation sows although a trend toward farrowing less stillborn pigs was observed in the sow groups fed diets containing 10 and 20% DFS. Second generation sows fed the diet containing 20% DFS farrowed less stillborn pigs in parity 1 but more than the other groups in parity 2. The number of stillborn pigs farrowed decreased after parity 1 in first generation sows but second generation sows farrowed more stillborn pigs in parity 2 than in parity 1.

The number of pigs weaned by first and second generation sows was not consistent with dietary treatment received. Additions of DFS to diets fed first generation sows reduced the number of pigs weaned while second generation sows fed 10 and 20% DFS weaned more pigs than sows fed the basal diet. First generation sows displayed a tendency to wean fewer pigs with each parity while second generation sows weaned more.

CONCLUSIONS

The long-term feeding of sewage sludge to female swine adversely effects many criteria used in evaluating reproductive performance. Breeding, farrowing, and rebreeding weights in both sow generations were lower in groups fed 10 and 20% sludge in diets. Lactation weight change was lower in sows fed the diets containing DFS. Gestation weight change was lowest in sow groups fed diets containing 10 and 20% additions of DFS.

First and second generation sows fed diets containing DFS farrowed fewer pigs while the number of live, stillborn, and weaned pigs was not influenced appreciably by treatment. The birth weights of pigs farrowed by both generations of sows did not differ with dietary treatment but pigs farrowed in parity 2 were heavier than those farrowed in other parities. First and second generation sows fed diets containing 10 and

20% DFS weaned lighter pigs when compared with sows fed the basal diet. Average daily gain (ADG) was also influenced by sow dietary treatment. In both sow generations, groups fed diets containing 10 and 20% DFS farrowed pigs that displayed reduced ADG from birth to weaning.

A comparison of the data indicates that overall reproductive performance was more diminished in second generation sows fed DFS in diets than in first. Weight changes in first and second generation sows and progeny weight data from each parity indicate that DFS is deficient in content, biological value, and/or availability of an essential nutrient(s).

The mean value of crude protein in 22 samples of DFS from the University of Florida sewage treatment plant reported by Beaudouin *et al.* (1980) shows that this nutrient partition is present in quantitatively attractive levels. However, crude protein, calculated from nitrogen content determined by the Kjeldahl method may be based partly on nonprotein nitrogen, nitrates, and nitrites. Swine do not utilize nonprotein nitrogen (NPN) efficiently (Pond and Maner, 1974) and trends in reproductive performance of sows in this study are similar to those of sows in other studies fed regimens in which dietary protein was restricted (Pond *et al.*, 1968; DeGeeter *et al.*, 1972; Hammell *et al.*, 1976). Additions of DFS to the basal diet appeared to dilute nutrient content and the detrimental effects on reproductive performance in female swine may have resulted in part from deficiencies in dietary protein.

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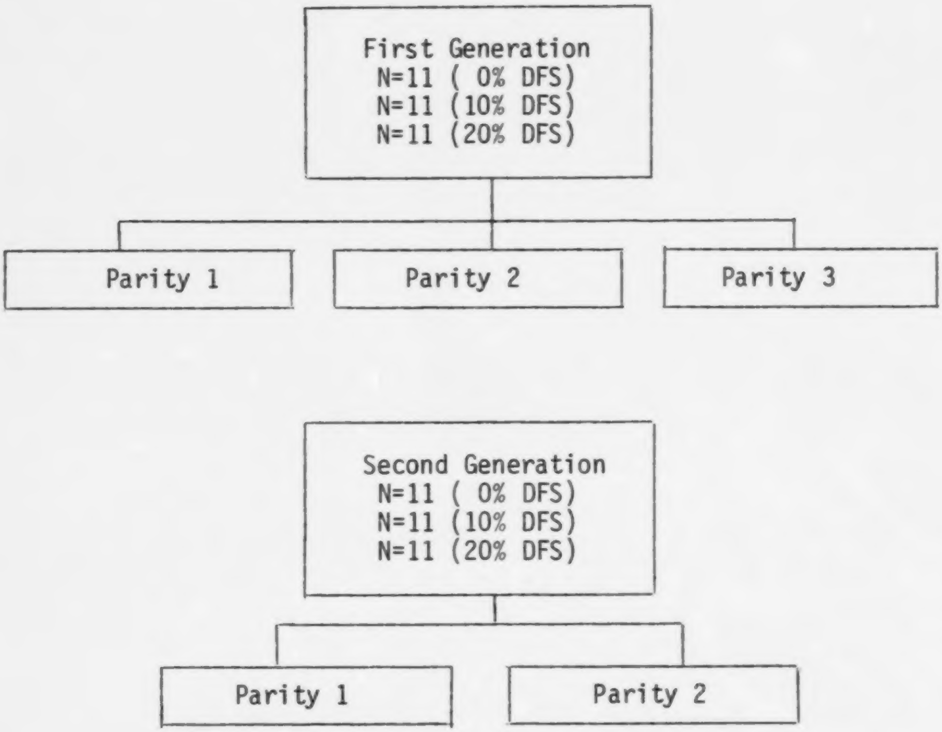
First Generation
N=11 (0% DFS)
N=11 (10% DFS)
N=11 (20% DFS)
Parity 1
Parity 2
Parity 3
Second Generation
N=11 (0% DFS)
N=11 (10% DFS)
N=11 (20% DFS)
Parity 1
Parity 2

FIGURE 1. DIAGRAM OF EXPERIMENTAL DESIGN USED TO EVALUATE EFFECT OF FEEDING DIGESTED SEWAGE SLUDGE ON LONG-TERM SOW REPRODUCTIVE PERFORMANCE

TABEL 15. GESTATION-LACTATION DIETS

Ingredient (%)	Diet 1	Diet 2	Diet 3
Corn	70.45	60.45	50.45
Sludge		10.00	20.00
Ground oats	10.00	10.00	10.00
Soybean meal	16.00	16.00	16.00
Defluorinated phosphate[1]	2.50	2.50	2.50
Iodized salt	0.50	0.50	0.50
Trace mineral premix[2]	0.05	0.05	0.05
Vitamin premix[3]	0.50	0.50	0.50
Antibiotic	+	+	+

[1] Provided courtesy of Occidental Petroleum Company, White Springs, FL

[2] Provides the following in ppm of diet: zinc, 200; iron, 100; manganese, 55; copper, 11; iodine, 1.5; and cobalt, 1.0. Courtesy of Calcium Carbonate Company, Quincy, IL

[3] Provides per lb of complete feed: vitamin A, 5000 IU; vitamin D, 2500 IU; vitamin E, 5 IU; riboflavin, 5 mg; pantothenic acid, 12 mg; niacin, 25 mg; choline chloride, 500 mg; and vitamin B_{12}, 20 mcg. Courtesy of Charles Pfizer and Company, Terra Haute, IN

TABLE 16. PROXIMATE ANALYSIS[a] OF DIGESTED SEWAGE SLUDGE FROM THE UNIVERSITY OF FLORIDA, GAINESVILLE SEWAGE TREATMENT PLANT

	Partition[b]					
Item	Air Dry Matter %	Crude Protein %	Crude Fiber %	Ether Extract %	Nitrogen free Extract %	Ash %
DFS[c]	91.9 (66-98)	25.9 (22-37)	.46 (.7-15)	.46 (.01-1.1)	40.6 (9.47)	28.8 (27-57)

[a] Data of Beaudouin *et al.*, 1980

[b] Percent dry matter

[c] Mean and range of 22 samples

TABLE 17. REPRODUCTIVE SUCCESS OF SOWS FED DIETS CONTAINING DIGESTED SEWAGE SLUDGE

		Percent Sewage Sludge (DFS) in Sow Diet					
		First Generation			Second Generation[a]		
	Parity	0	10	20	0	10	20
Sows Bred	1	11	11	11	11	11	11
	2	11	11	11	11	11	11
	3	11	11	11	-	-	-
Sows farrowed	1	11	9	9	8	7	7
	2	10	11	10	8	6	7
	3	11	10	10	-	-	-
Gestation length (days)	1	114.7	114.2	114.4	115.6	114.1	114.0
	2	114.6	114.8	116.1	113.8	114.5	114.0
	3	114.6	114.1	115.3	-	-	-

[a] Criteria for first and second generations do not differ ($P < 0.05$)

TABLE 18. WEIGHT RESPONSE OF SOWS FED DIETS CONTAINING DIGESTED SEWAGE SLUDGE

		Sewage Sludge (DFS) in Sow Diet (%)							
		First Generation				Second Generation[a]			
Criterion	Parity	0	10	20	(Avg)	0	10	20	(Avg)
Average body weight (kg)									
Breeding	1	76.5	77.7	79.6	(77.8)^e	106.5	99.3	100.6	(102.1)^c
	2	144.1				156.6	144.0	134.3	(144.9)^b
	3	157.2	156.4	145.8	(153.1)^f	-	-	-	-
	Avg	125.9	127.2	119.0		131.6^h	121.7^i	117.5^i	
Farrowing	1	169.5^h	161.7^h	151.0^i	(160.7)^b	176.0^h	154.4^i	150.3^i	(160.2)^c
	2	191.6^h	190.0^h	162.3^i	(181.3)^b	198.3^h	167.1^i	152.6^i	(172.7)^b
	3	177.6^h	171.5^{hi}	160.6^i	(169.9)^c	-	-	-	-
	Avg	179.6^h	174.4^{hi}	158.0^i		187.2^h	160.8^i	151.5^i	
Re-breeding	1	144.1	147.3	131.7	(141.0)^c	157.4^h	134.9^i	128.4^i	(140.2)
	2	157.2	156.4	145.8	(153.1)^b	165.1^h	143.5^i	141.8^i	(150.1)
	3	161.9^h	152.1^{ghi}	153.5^i	(155.8)^b	-	-	-	-
	Avg	154.4^h	151.9^{ghi}	143.7^i		161.3^j	139.2^k	135.1^k	
Lactation Change	1	-25.4^h	-12.1^h	-19.3^i	(-18.9)^{bc}	-18.6^h	-19.4^{hi}	-21.9^i	(20.0)
	2	-34.5^h	-33.6^h	-16.5^i	(-28.2)^b	-33.2^h	-23.6^{hi}	-10.8^i	(22.5)
	3	-15.8	-19.5	-14.9	(-16.7)^c	-	-	-	-
	Avg	-25.2	-12.7	-16.9		-25.9	-21.5	-16.4	
Gestation Change	1	93.0^h	83.3^{hi}	71.4^i	(82.6)^e	67.1^h	56.0^i	52.5^i	(58.5)^e
	2	45.9^h	38.1^{hi}	28.6	(37.5)^f	38.2^h	17.3^i	14.6^i	(23.4)^f
	3	20.6^j	14.8^{jk}	14.8^k	(16.7)^g	-	-	-	-
	Avg	53.2^j	45.4^{jk}	38.3^k		52.7^j	36.7^k	33.6^k	

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[a] Second generation sows were offspring from the second parity of first generation sows.

[b,c,d] Means within columns not followed by the same superscript are significantly different ($P < 0.05$)

[e,f,g] Means within columns not followed by the same superscript are significantly different ($P < 0.01$)

[h,i] Means within rows not followed by the same superscript are significantly different ($P < 0.05$)

[j,k] Means within rows not followed by the same superscript are significantly different ($P < 0.01$)

TABLE 19. EFFECTS OF SOW DIETS CONTAINING DIGESTED SEWAGE SLUDGE ON CORRESPONDING PROGENY MORTALITY

		Sewage Sludge in Sow Diets (%)							
		First Generation				Second Generation[a]			
Criterion	Parity	0	10	20	(Avg)	0	10	20	(Avg)
Pigs farrowed/ litter (no.)	1	10.6	9.4	9.7	(9.9)	8.5^{f}	8.3^{g}	11.3^{fg}	(9.4)^{c}
	2	10.6	10.0	9.1	(9.9)	13.9^{f}	10.8^{g}	12.9^{fg}	(12.5)^{b}
Total	3	9.0	10.9	8.1	(9.3)	-	-	-	-
	Avg	10.1	10.1	9.0		11.2^{d}	9.6^{d}	12.1^{e}	
Live	1	9.1	8.1	9.0	(8.7)	7.3	7.7	10.9	(8.6)^{c}
	2	9.9	9.6	8.3	(9.3)	12.3	10.3	10.3	(11.0)^{b}
	3	7.6	10.1	7.8	(8.5)	-	-	-	-
	Avg	8.9	9.3	8.4		-	-	-	
Still-born	1	1.3	1.2	.7	(1.1)	1.3^{fg}	.6^{g}	.4^{f}	(.7)^{e}
	2	.7	.4	.8	(.6)	1.8^{fg}	.5^{g}	2.6^{f}	(1.6)^{d}
	3	1.0	.8	.3	(.7)	-	-	-	-
	Avg	1.0	.8	.6		1.6	.6	1.5	
Pigs weaned	1	7.9	6.9	7.2	(7.3)	4.3	5.9	7.6	(5.9)
	2	8.0	6.3	6.3	(6.9)	7.4	8.7	6.9	(7.7)
	3	6.5	6.5	6.6	(6.5)	-	-	-	-
	Avg	7.5	6.5	6.7		5.9	7.3	7.3	

---

[a] Second generation sows were offspring from the second parity of first generation sows.

[b,c] Means within columns not followed by the same superscript are significantly different ($P < 0.01$)

[d,e] Means within columns not followed by the same superscript are significantly different ($P < 0.05$)

[f,g] Means within rows not followed by the same superscript are significantly different ($P < 0.05$)

TABLE 20. BIRTH TO WEANING[a] PERFORMANCE OF PROGENY FROM SOWS FED DIGESTED SEWAGE SLUDGE

		Sewage Sludge in Sow Diet (%)							
		First Generation				Second Generation[b]			
Criterion	Parity	0	10	20	(Avg)	0	10	20	(Avg)
Average body wt (kg)									
Birth	1	1.41	1.49	1.34	(1.41)^{d}	1.41	1.44	1.44	(1.43)
	2	1.55	1.58	1.47	(1.54)^{c}	1.51	1.62	1.66	(1.59)
	3	1.39	1.39	1.37	(1.39)^{d}	-	-	-	-
	Avg	1.45	1.49	1.39		1.46	1.53	1.55	
Weaning	1	5.39	5.23	4.72	(5.14)	5.06	4.95$_{f}$	4.67$_{f}$	(4.89)^{d}
	2	5.11	4.87	5.24	(5.07)	6.22^{e}	4.98^{f}	5.15^{f}	(5.52)^{c}
	3	5.54	4.94	5.18	(5.24)	-	-	-	-
	Avg	5.35	5.01	5.05		5.64^{e}	4.97^{f}	4.91^{f}	
ADG	1	.187	.179	.168	(.179)	.172	.166$_{f}$	.154$_{f}$	(.164)
	2	.218	.165	.163	(.170)	.218^{e}	.165^{f}	.163^{f}	(.182)
	3	.193	.167	.178	(.181)	-	-	-	(.182)
	Avg	.199	.170	.170		.195^{e}	.166^{f}	.159^{f}	

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[a] 21 days post partum

[b] Second generation sows were the offspring of the second parity of first generation sows

[c,d] Means within each column not sharing a common superscript are significantly different ($P < 0.05$)

[e,f] Means within each row not sharing a common superscript are significantly different ($P < 0.05$)

SWINE - OTHER HEALTH EFFECTS

G. T. Edds, C. F. Simpson, J. A. Popp, O. Osuna, K. E. Ferslew,
R. L. Suber, and K. L. Kelly

Research was initiated in growing and breeding animals to determine whether inclusion of various levels of urban sewage sludge in the rations would adversely affect animal performance or gestation or result in hazardous residues of metals or other toxicants that might affect human health. The data reported in this chapter will describe the variety of trials that influenced the other health effects in swine.

Several parameters were included in the early trials on possible health hazards of including 10 and 20% dried University of Florida sewage sludge (DFS) in the rations of swine. It was suspected that bacteria and/or viruses might persist in the DFS, thus, total white cell and differential white cell counts were done on blood samples taken at the beginning and at intervals throughout the feeding trials. Serum enzyme tests were performed to identify infection or migratory damage from pathogens or parasites. Performance of prothrombin times would give evidence of liver damage associated with toxicants, especially endotoxins or aflatoxins. Blood and tissue analyses were performed for metals or drugs suspected as hazardous residues.

Newborn pigs were sacrificed and examined for normal blood levels of cells and enzymes or for residue hazards transmitted from their dams through their milk.

At the time of initiation of each trial and monthly thereafter, the animals were bled for cellular and enzyme determinations, packed cell volumes; blood samples were also collected for bacteriological and pesticide evaluations. In addition, feed and fecal samples were collected for similar studies; the latter was also checked for type and degree of parasite infection.

Earlier trials incorporating dried University of Florida sludge (DFS) into swine rations by Beaudouin *et al.*, and Hammell *et al.*, suggested that accumulation or toxic effects developed from chronic exposure to rations containing some elements present in the 10 or 20% dried sludge. Therefore, a series of research trials were performed to determine which factor(s) in sludge might be responsible for the delayed toxicity.

EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 287)
TYPING GUIDE SHEET

SECTION A. Feeding Trials of Dried Urban Sludge and the Equivalent Cadmium Level in Swine. O. Osuna, G. T. Edds, J. A. Popp, J. Monegue, and K. E. Ferslew

SECTION B. Toxicology of Aflatoxin B_1, Warfarin, and Cadmium in Young Pigs. O. Osuna and G. T. Edds

SECTION C. High Performance Liquid Chromatographic Determination of Sulfonamides by Ionic Suppression. R. L. Suber and G. T. Edds
Abstract

SECTION D. Comparison of the Pharmacokinetics of Sulfisoxazole in Humans and Two Monogastric Species. R. L. Suber, C. Lee, G. Torosian, and G. T. Edds
Abstract

SECTION E. Comparison of the Potential Toxicity of Bilirubin in Humans and Two Monogastric Species after a Single Administration of Sulfisoxazole. R. L. Suber, J. C. Gudat, and G. T. Edds
Abstract

A. FEEDING TRIALS OF DRIED URBAN SEWAGE AND THE EQUIVALENT CADMIUM LEVEL IN SWINE

ABSTRACT

Toxicity from feeding dried sewage sludge included in a normal swine starter ration may occur from a deficiency of available protein or other essential nutrients, or from the accumulation of hazardous chemical residues.

One lot of sewage sludge from Chicago was found to contain high levels of cadmium (165 ppm). This trial compared the effects of feeding weanling pigs a starter ration containing 50% dried, activated, Chicago sewage sludge with a standard 18% crude protein basal diet, plus 83 ppm cadmium, for 9 weeks.

Forty-eight 4 week-old, hybrid pigs were allotted to 2 replicate experiments of 2 treatment groups each. Body weight, feed consumption, and blood samples were determined weekly including PCV, RBC, WBC, MCV, Hb, and serum levels of 4 enzymes, AP, γGT, GOT, GPT. Feed and fecal samples were collected weekly and analyzed for cadmium content. Tissue samples were provided at the slaughter time on days 38, 42, and 56 for metal analyses and pathologic evaluations.

Depressed growth and feed consumption were evident in pigs consuming 50% Chicago sludge and 83 ppm of cadmium.

Cadmium exposure induced microcytic and hypochromic anemia. At necropsy, pale, white muscles and kidneys were observed.

INTRODUCTION

In swine production with modern feeding and management conditions, cadmium toxicity is relatively rare. However, borderline toxicities are possible where animals ingest recycled waste materials, such as urban sewage sludge, in which cadmium may be concentrated (Neathery and Milton, 1976). Two percent sludge has been found to provide a satisfactory source of vitamin B_{12} for the pig. Toxicity from feeding dried sewage sludge included in a normal swine starter ration, may occur from a deficiency of available protein or other essential nutrients, or from the accumulation of hazardous chemical residues (Edds *et al.*, 1978).

Cadmium interferes with the functioning of necessary elements such as zinc in enzyme systems (Flick *et al.*, 1979; Friberg *et al.*, 1971). Also, a lethal dose of cadmium may inhibit mitochondrial oxidative

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TYPING GUIDE SHEET

phosphorylation in rat liver and may be directly correlated with death (Southard *et al.*, 1974). General clinical symptoms of cadmium toxicity in mammals include anemia, retarded testicular development or degeneration, enlarged joints, scaly skin, liver and kidney damage, reduced growth and increased mortality (Miller, 1971; Powell *et al.*, 1964).

Studies of animal or human exposure to cadmium suggest that neither blood nor urine is a reliable indicator of total body burden of cadmium. Blood is unsuitable because the sojourn of cadmium in this tissue is brief, and its concentration in blood is extremely low (Petering *et al.*, 1973). Consequently, blood cadmium data have little diagnostic value. After absorption, most cadmium is transported in plasma bound to gamma-globulin (Shakin and Lerces, 1972). However, some may be bound with hemoglobin or metallothionein in erythrocytes (Carlson and Friberg, 1957).

Generally, highest cadmium concentrations are in kidney, followed by liver (Friberg *et al.*, 1971; Miller *et al.*, 1968; Neathery *et al.*, 1974). Muscle is well protected from ingested cadmium (Neathery and Miller, 1976).

The most notable finding upon examination of a reported case of cadmium toxicity in swine was an extreme anemic condition (Alber, 1963). It was found that oral or injected iron offered protection against cadmium induced anemia in swine (Pond *et al.*, 1973).

Recently, studies on the use of animal waste and sewage sludge for animal feeds have been of interest. One lot of sewage sludge from Chicago was found to contain high levels of cadmium (165 ppm). This trial compared the effects of feeding weanling pigs a starter ration containing 50% dried, activated, Chicago sewage sludge or a standard 18% crude protein basal diet, plus 83 ppm cadmium, for 9 weeks.

MATERIALS AND METHODS

Forty-eight, 4-6 week old, hybrid pigs were allotted to 2 replicate experiments of 2 treatments each, 12 pigs per group. The first of the 2 concurrent experiments included 12 barrows in both the control and the 83 ppm cadmium groups. The second experiment consisted of 6 males and 6 females in both the control and 50% Chicago sludge groups.

The pigs were fed a basal swine starter ration with an 18% crude protein basal diet (Table 1) as the control and as the ration to which either the cadmium was added in the form of $CdCl_2$ at an 83 ppm level or the dried, activated, Chicago sewage sludge was added at 50% level.

All rations were tested weekly and found negative for aflatoxin B_1[a]. Feed and water were provided *ad libitum* for 9 weeks. Pigs were housed by treatment in concrete floored and cement block pens with automatic

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[a] Minicolumn Technique, Southern Marketing and Nutrition Research Division, Agricultural Research Service, U.S.D.A., P.O. Box 19687, New Orleans, LA.

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U.S. GOVERNMENT PRINTING OFFICE 1980-660-630
TYPING GUIDE SHEET

watering devices and standard pig self-feeders. Floors were washed daily or when necessary to provide sanitary conditions. Feed was checked daily and added when needed.

TABLE 1. BASAL DIET CONSUMPTION*

Ingredient	%
Yellow corn meal	71.8
Soybean meal	25.0
Dyna-fos	1.7
Limestone	0.8
Iodized salt	0.25
Trace mineral mix (ccc)	0.10
Vitamin premix (UF)	0.10
ASP-250	0.25

[*] Swine Feed Starter Ration, 18.71% protein, medicated, University of Florida.

Body weight (BW), blood, and fecal samples were taken daily. Blood samples were procured by anterior vena cava puncture. Blood samples were collected in tubes[b] with disodium edetate for white blood cell counts (WBC), red blood cell counts (RBC), packed cell volumes (PCV), mean corpuscular volumes (MCV), and hemoglobin (Hb). Serum samples were obtained for alkaline phosphatase (AP), gamma-glutamyl transpeptidase (γGT), glutamate pyruvate transaminase (SGPT), & glutamate oxaloacetate transaminase (SGOT) analyses. Enzyme analyses were performed with a centrifugal analyzer[c] using standard procedures, reagents, and controls[d].

Four pigs from each treatment group were slaughtered on weeks 4, 6, and 9 (days 28, 42, and 56, respectively). Tissue samples collected for histopathology and metal analyses included: kidney, liver, muscle, heart, lung, spleen, stomach, small intestine, and testicle or ovary in the second section of the trial. Liver samples were obtained from the center hepatic lobe, left kidney, and muscle from the left ham.

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[b] Baltimore Biological Laboratory, Division of B-D Laboratories, Inc., Baltimore, MD.

[c] Gemsaec Centrifugal Analyzer, Electro Nucleonics, Inc., Fairfield, NJ.

[d] Boehringer Mannheim Corp., Norcross, GA.

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Feed, tissue, and blood samples were slow-digested with hydrochloric acid. Residues were then detected by atomic absorption[e] using standard procedures[f]. The various samples were tested for cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), molybdenum (Mo), nickel (Ni), lead (Pb), selenium (Se), and zinc (Zn).

Statistical analyses were performed by application of an analyses of variance (ANOVA) to the data. Testing for significant differences on any particular day between groups was done by "T" test analysis[g].

RESULTS

Performance

Depressed growth and feed consumption were evident in the pigs consuming 83 ppm Cd ($P < 0.05$) in the diet. The control pigs of the first experiment gained 0.37 pounds (lbs) more per day on the average than those consuming Cd. Feed consumption for the same experiment averaged 0.28 lbs per day less for the pigs receiving Cd. A decrease in feed efficiency was also noted. The pigs receiving cadmium required 0.21 lbs of feed more per lb of gain than those in the controls.

The pigs on the second experiment consuming 50% dried, activated, Chicago sewage sludge (DCS) did not perform as well as the control pigs. Weight gains of all pigs in the 50% DCS group were lower ($P < 0.01$) than those in the control group as described by Edds *et al.*, 1978.

Hematology

The RBC, MCV, Hb, and PCV of the cadmium pigs declined steadily and significantly from days 21 through 56. An extreme microcytic, hypochromic anemia was observed by day 42 of the trial ($P < 0.01$). There was no significant difference in the WBC levels in this first experiment.

The RBC, MCV, PCV, and Hb of the 50% DCS group of the second experiment paralleled the values of the control group throughout the experiment with no significant differences. The WBC counts of this 50% DCS group were significantly higher ($P < 0.01$) at the sixth week.

Serum Enzyme Levels

None of the 4 serum enzymes measured in the first experiment (AP, γGT, GOT, and GPT) (Table 2) showed differences between treatments.

In the second experiment with 50% DCS, there was significant increase in GOT (Table 3) at weeks 3, 5, and 7. There were no significant changes with the other enzymes (AP, γGT, and GPT).

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[e] Perkin-Elmer Atomic Absorption Unit.

[f] IFAS Soils Department, University of Florida, Gainesville, FL.

[g] IFAS Statistics Department, University of Florida, Gainesville, FL.

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TYPING GUIDE SHEET

Metal Analyses

In the first experiment, Cd was at a higher concentration both in the liver ($P < 0.0001$) and kidney ($P < 0.0001$) in the pigs consuming 83 ppm of cadmium. No Cd was detected in blood or muscle of the control and Cd treated groups. Copper was significantly higher in the kidney ($P < 0.0037$) but lower in the muscle ($P < 0.048$), liver and blood ($P < 0.034$) in the pigs consuming 83 ppm of Cd. Zinc was significantly higher in the kidney ($P < 0.0001$) but lower in the liver ($P < 0.0006$) and muscle ($P < 0.0221$) tissues of the 83 ppm Cd treated group. Iron was significantly decreased in the liver ($P < 0.0001$), kidney ($P < 0.0001$) and blood ($P < 0.0001$) in the pigs treated with 83 ppm Cd. There were no differences in the levels of Co, Hg, Pb, Ni, or Cr in the liver, kidney, muscle, and blood between the control and 83 ppm Cd groups. Molybdenum was significantly higher ($P < 0.017$) in the kidney but not significantly different in liver, muscle, and blood of the 83 ppm Cd treated pigs (Table 5).

In the second experiment, Cd was at a greater concentration in the liver ($P < 0.001$) and kidney ($P < 0.001$) in the pigs consuming 50% DCS. However, the concentrations of Cd in the liver and kidney of the 50% DCS group were lower than the concentrations in the same organs of the 83 ppm Cd treated group. No Cd was found in either blood or muscle of the control and 50% DCS treated groups. Copper concentration was significantly increased in the kidney ($P < 0.001$) in the 50% DCS treated group but no differences were determined between groups in the liver, muscle, and blood. Zinc was significantly increased in the kidney ($P < 0.001$) but lower in the liver ($P < 0.0276$) and muscle ($P < 0.037$) of the 50% DCS treated group. Iron was significantly increased in the liver ($P < 0.0161$) but was lower in the muscle ($P < 0.049$) of the 50% DCS pigs. No iron differences were observed in the kidney or blood of the 2 control and 50% DCS groups. There were significantly increased concentrations of Hg ($P < 0.015$), Ni ($P < 0.0003$), and Cr ($P < 0.018$) in the kidney of the pigs treated with 50% DCS. There were no differences in the concentrations of Fe, Co, Mo, and Pb in the kidneys of the control and 50% DCS groups. Chromium was significantly increased in the liver ($P < 0.0274$) of the 50% DCS treated group. No significant differences between groups were observed in the liver and muscle of Cu, Co, Mo, Hg, and Pb. No significant differences of Cr, Zn, Co, Mo, Pb, Hg, and Ni in blood occurred between groups. More significant levels of muscle Zn levels ($P < 0.024$) and blood copper levels ($P < 0.0002$) were observed in the males than in the females (Table 6).

Pathology

The pathologic examination of heart, liver, intestine, kidney, and lung tissues collected at slaughter from the 4 treatment groups revealed only minor lesions. The tissues from the control pigs were normal except the intestinal tracts showed increased eosinophils in the lamina propria. The intestines from the animals receiving the Cd supplemented ration were normal. The heart tissue of one of the pigs in the Cd supplemented group

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(PREVIOUSLY CIN. EPA FORM 287)
U.S. GOVERNMENT PRINTING OFFICE 1980-660-639
TYPING GUIDE SHEET

had a small zone of focal myocarditis and a lymphoid nodule protruding into a bronchus. Two of the other pigs in this group also had focal mononuclear cell infiltration into the lung tissue.

The tissues, liver, lung, and intestinal tract of the group on 50% Chicago sludge showed acute cholangitis, hepatic cytoplasmic vacuolation, mononuclear cell infiltration into alveolar tissues; foci of eosinophils were localized in the intestinal lamina propria.

No lesions were observed in the kidney tissues in any of the groups.

DISCUSSION

Performance

Depressed growth and feed consumption were evident in pigs consuming either rations containing 83 ppm of Cd or in pigs with 50% Chicago sludge. Toxicity from feeding dried sewage sludge included in a normal swine starter ration may occur from a deficiency of available protein or other essential nutrients, or from the accumulation of hazardous chemical residues (Edds *et al.*, 1978).

Hematology

When the control values of blood between the 2 replications are compared, important conclusions can be made: (1) RBC counts in both control groups fall into the same range between 6.3 and 7.3 millions/mm^3; (2) MCV values in both control groups fall into the same range between 44 to 56 cubic microns; (3) Hb values in both control groups fall into the same range between 11.1 to 13.2 gm%; (4) PCV values of both control groups fall into the same range between 35 and 41. Therefore, the conditions of the 2 replications may be considered normal physiologic standards for swine between 4 to 15 weeks of age.

The microcytic hypochromic anemias are specific for iron deficiency or failure to utilize iron. Chronic blood loss or iron, copper, and pyridoxine deficiencies must be considered (Schalm, 1965). After absorption, most Cd is transported in plasma bound to gamma-globulin (Shaik and Lerces, 1972); however, some may be bound with hemoglobin or metallothionein in red blood cells (Carlson and Friberg, 1957).

Our results agree with Alber (1963) that cadmium toxicity in swine induces anemia and with Pond *et al.* (1973) that oral iron offers protection against cadmium induced anemia in swine. The last statement can be understood when the Fe concentrations of the diets are considered. There was a high concentration of Fe (8,846.9 ppm) in the diets of the 50% Chicago sewage sludge treated group. The concentrations of Fe in the Cd treated group was similar to the control group of the first experiment. Therefore, some types of sludge could be considered as a source of iron for young pigs to correct anemia induced by Fe deficiency.

Serum Enzyme Levels

The enzyme values are in agreement with histopathologic results. There were no indications of liver damage induced by the 83 ppm of Cd in the diets.

The increased levels of GOT and the high WBC counts in the 50% DCS group may have resulted from histopathologic changes, characterized by central lobular necrosis and vacuolation as well as acute cholangitis, in the liver of some of the pigs.

Metal Analysis

We agree with Petering *et al.* (1973) that blood is unsuitable for determination of Cd concentrations and has little diagnostic value. Neither is muscle tissue since Cd levels are not detectable at all and our 0 values are in agreement with several other reports (Neathery and Miller, 1976). Therefore, animal muscle residue levels would pose no hazard for human health.

Higher Cd concentrations were found in the kidney, followed by the liver as reported by others (Neathery and Miller, 1976; Friberg *et al.*, 1971) in both the 83 ppm Cd and the 50% DCS groups. Probably, other elements such as Fe, Zn, and Cu present at high concentration in the 50% DCS feed (Table 4), decrease the absorption of Cd in the gastrointestinal tract. This may explain the lower concentrations of Cd in tissues in this group as compared to the group receiving 83 ppm Cd. In fact, tissue Zn levels were elevated in the 83 ppm Cd treated pigs which is in agreement with Powell *et al.* (1964).

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(PREVIOUSLY CIN. EPA FORM 267)
TYPING GUIDE SHEET

TABLE 2. SERUM ENZYME LEVELS FOR CADMIUM TRIALS

Weeks	AP (iu/l) Control	AP (iu/l) 83 ppm Cd	γGT Control	γGT 83 ppm Cd	GOT Control	GOT 83 ppm Cd	GPT Control	GPT 83 ppm Cd
1	673.1	713.7	15.2	13.3	31.5	31.0	50.3	56.1
2	827.7	812.3	27.2	25.6	25.1	33.4	43.5	50.2
3	856.1	771.0	18.7	17.7	97.9	88.3	43.2	44.0
4	756.3	685.3	14.9	16.1	69.7	66.6	50.0	56.3
5	646.0	601.2	16.5	17.6	36.6	43.4	56.4	57.6
6	703.1	601.0	15.5	14.8	37.1	35.2	29.0	35.2
7	457.5	529.3	13.3	14.9	19.7	33.9	36.6	46.7
8	507.2	601.3	15.2	18.4	12.0	25.6	21.1	31.9
9	564.2	580.3	10.1	13.2	21.0	12.0	31.1	23.4

TABLE 3. SERUM ENZYME LEVELS FOR CHICAGO SEWAGE SLUDGE TRIALS

	AP (iu/l)		γGT		GOT		GPT	
Weeks	Control	50% sludge	Control	50% sludge	Control	50% sludge	Control	50% sludge
1	504.8	570.8	9.0	13.3	11.4	15.3	34.7	35.1
2	604.3	561.3	13.7	16.7	24.7	27.3	32.6	20.5
3	662.8	557.2	15.7	12.8	36.1	57.3*	39.2	40.6
4	614.9	518.5	13.7	14.9	24.3	25.1	28.2	28.4
5	556.3	644.7	12.9	15.3	23.7	44.5*	29.6	25.8
6	640.9	526.6	14.9	18.2	18.4	32.0*	31.2	25.3
7	522.4	402.3	15.9	16.0	17.9	42.3*	26.0	24.8
8	571.0	423.5	13.6	10.4	16.0	40.1	22.8	13.4
9	484.3	468.8	12.2	12.6	22.7	24.0	23.3	20.6

* $P < 0.01$

TABLE 4. AVERAGE METAL VALUES IN FEED (PPM)

	Control	83 ppm Cd	Control	50% Sewage Sludge
Cd	0.36	78.6[5]	0.12	147.3[2]
Cu	13.9	13.4	13.9	331.6
Fe	377.7	373.5	237.2	8,846.9[3]
Co	0.62	0.62	0.69	6.16
Zn	209.8	190.6	183.4	773.7[1]
Pb	1.09	0.62	1.66	129.9
Ni	1.71	1.36	2.75	123.8[4]
Cr	2.65	2.27	2.76	696.4

[1] $P < 0.0001$

[2] $P < 0.0004$

[3] $P < 0.0006$

[4] $P < 0.0007$

[5] $P < 0.01$

TABLE 5. AVERAGE MEAN VALUES OF METAL CONCENTRATIONS IN TISSUES OF YOUNG PIGS TREATED WITH 83 PPM $CdCl_2$ IN THE DAILY DIETS

	LIVER		KIDNEY		MUSCLE		BLOOD	
	Control	83 ppm Cd	Control	83 ppm Cd	Control	83 ppm Cd	Control	83 ppm Cd
Cd	0.00	12.98[1]	0.00	61.95[1]	0.00	0.00	0.00	0.00
Cu	7.55	6.35	8.93	15.08[5]	0.81	0.67[6]	1.39	1.20[7]
Fe	118.81	23.15[1]	31.28	16.54[1]	7.53	7.23	478.5	273.8[2]
Zn	53.89	41.25[2]	20.62	34.08[1]	14.01	10.00[4]	8.26	6.98[7]
Mo	1.25	1.30	0.73	0.78[3]	0.00	0.00	0.00	0.00
Co	0.00	0.08	0.03	0.03	0.03	0.00	0.00	0.00
Pb	0.00	0.00	0.00	0.00	0.03	0.03	0.00	0.00
Hg	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00
Ni	0.00	0.00	0.11	0.02	0.00	0.00	0.00	0.00
Cr	0.00	0.03	0.00	0.09	0.00	0.00	0.00	0.00

[1] $P < 0.0001$

[2] $P < 0.0006$

[3] $P < 0.0017$

[4] $P < 0.0022$

[5] $P < 0.0037$

[6] $P < 0.0048$

[7] $P < 0.03$

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TABLE 6. AVERAGE MEAN VALUES OF METAL CONCENTRATIONS IN TISSUES OF YOUNG PIGS FED 0 AND 50% CHICAGO SEWAGE SLUDGE (PPM)

	Control	50% DCS	Control	50% DCS	Control	50% DCS	Control	50% DCS
Cd	0.00	3.15[1]	0.00	23.49[1]	0.00	0.00	0.02	0.02
Cu	5.30	5.90	6.60	10.70[1]	0.800	0.831	1.05	1.12
Fe	133.67	163.24[4]	41.03	41.03	10.40	8.30[8]	442	440.60
Zn	57.80	51.30[7]	24.30	37.40[1]	13.99	12.49[7]	4.64	4.58
Mo	1.00	0.92	0.70	0.79	0.02	0.02	0.00	0.00
Co	0.03	0.00	0.03	0.05	0.00	0.00	0.01	0.03
Pb	0.00	0.05	0.05	0.00	0.00	0.00	0.00	0.06
Hg	0.00	0.05	0.014	0.368[3]	0.00	0.00	0.00	0.00
Ni	0.00	0.00	0.021	0.565[2]	0.00	0.00	0.04	0.04
Cr	0.056	0.165[6]	0.214	0.380[5]	0.00	0.00	0.15	0.11

[1] $P < 0.0001$

[2] $P < 0.0003$

[3] $P < 0.0015$

[4] $P < 0.0161$

[5] $P < 0.0180$

[6] $P < 0.0274$

[7] $P < 0.0376$

[8] $P < 0.049$

B. TOXICOLOGY OF AFLATOXIN B_1, WARFARIN, AND CADMIUM IN YOUNG PIGS

ABSTRACT

The objectives of this experiment were to compare the toxic effects of aflatoxin B_1, a dihydrofuranocoumarin, and warfarin, a 3-(α-acetonyl-benzyl) -4 hydroxycoumarin; and also to determine whether an additive effect from either aflatoxin B_1 or warfarin occurs when cadmium is present in higher than normal levels in the diets of young pigs.

Thirty-six healthy weaned barrows, mixed breed, averaging 9 kg of body weight, were assigned at random to 6 treatment groups, 6 pigs per group: Group I - negative control; Group II - 0.2 mg/kg of aflatoxin B_1; Group III - 0.2 mg/kg of warfarin; Group IV - 83 µg/g of cadmium diet; Group V - 83 µg/g of cadmium diet plus 0.2 mg/kg of aflatoxin B ; Group VI - 83 g/g of cadmium diet plus 0.2 mg/kg of warfarin. Groups II, III, V, and VI received 5 daily doses of the chemical in gelatin capsules during the fifth week of the experiment and the effects were followed for 10 days. Cadmium (Cd) was provided daily through the diets (given as cadmium chloride) during the 40 days of the experiment.

The body weight loss ($P < 0.0065$) in the aflatoxin B_1 group was associated with decreased feed consumption. Significantly lower values in serum total protein ($P < 0.0378$), alpha globulin ($P < 0.0133$) beta globulin ($P < 0.00119$), gamma globulin ($P < 0.05$), and plasma fibrinogen ($P < 0.0279$) were induced by aflatoxin B_1.

Significantly increased values of alkaline phosphatase ($P < 0.016$), sorbitol dehydrogenase ($P < 0.003$), and aspartate aminotransferase ($P < 0.05$) were determined 48 hours after initiation of the dosing with aflatoxin B_1 and correlated with hepatic fatty infiltration and vacuolation through all lobules.

Warfarin was more effective in producing earlier and higher values in prothrombin time ($P < 0.001$) and activated partial thromboplastin time ($P < 0.007$) than aflatoxin B_1 by the second day after initiation of dosage.

Depressed growth, feed consumption, feed efficiency, white blood cell counts ($P < 0.0056$), gamma globulin values ($P < 0.0018$), and an extreme microcytic hypochromic anemia were evident in the pigs consuming 83 µg/g Cd in the diet.

The Cd concentrations were highest in kidney, 42.9 µg/g, next in liver, 7.92 µg/g, and correlated well with the loss of iron from the kidney ($P < 0.0001$) and liver ($P < 0.0001$) in pigs treated with 83 µg/g Cd diets.

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Cadmium may have blocked the liver microsomal enzyme system and prevented the activation of aflatoxin B_1 to a toxic anticoagulant metabolite. On the other hand, the blockade prevented the inactivation of warfarin, enhancing its anticoagulant effects. This blockade was demonstrated through clinical signs, hematologic, electrophoretic and clinical chemistry changes, as well as gross and histopathologic lesions. It is concluded that there is an inhibitory effect on the toxicities of aflatoxin B_1 while there is an enhancing synergistic effect with warfarin when Cd is present in the diets of young pigs at 83 μg/g.

C. HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATIONS OF SULFONAMIDES BY IONIC SUPPRESSION

ABSTRACT

A high pressure liquid chromatography procedure is reported for extraction and quantitation of 8 sulfonamides in stock solutions and *in vitro* plasma samples. This assay consists of a single, one-step extraction of sulfonamides from plasma and is sensitive to 10.0 ng/ml at 254 nm without additional concentration of the sample. Four sulfonamides (sulfamerazine, sulfamethazine, sulfapyridine, and sulfathiozole) were separated from the plasma matrix by either mobile phase regardless of pH. The sulfonamides with the highest pKa, sulfanilamide (10.5) and sulfaguanidine (11.3), were only separable from plasma in a 50% water/50% methanol mobile phase at pH 7.45. The sulfonamide with the lowest pKa, sulfisoxazole (4.9), and its metabolite, acetylsulfisoxazole (N^4), were separated from plasma by either mobile phase, 50/50 or 60/40 water/methanol, when acetate buffer reduced the pH to 4.00. Standard concentration curves of peak height were the most sensitive at 254 nm when a 60% water/40% methanol mobile phase at pH 4.00 was used. Sulfanilamide and sulfaguanidine were the most responsive to ultraviolet quantitation at 254 nm regardless of ionic suppression or polarity of the mobile phase.

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D. COMPARISON OF THE PHARMACOKINETICS OF SULFISOXAZOLE IN HUMANS AND TWO MONOGASTRIC SPECIES

ABSTRACT

The pharmacokinetic profile of sulfisoxazole in dogs and swine was found to be biexponential; humans were observed to have a single compartment for this sulfonamide. The mean extrapolated unbound, unmetabolized serum concentration of sulfisoxazole in the first compartment was 189.42 ± 38.45 μg/ml in dogs and 245.07 ± 44.88 μg/ml in swine, in the second compartment, the extrapolated concentration was 2.56 ± 0.39 μg/ml in dogs and 0.423 ± 0.088 in swine. The mean disposition rate from the first or distribution compartment was 0.1726 ± 0.0604 hours^{-1} in dogs and faster in swine, 0.5368 ± 0.0362 hours^{-1}. The mean disposition rate from the second or elimination compartment was much slower in dogs and swine, 0.206 ± 0.0014 hours^{-1}, and 0.0153 ± 0.0043 hours^{-1}, respectively. Maximum serum acetylsulfisoxazole, the N^4 metabolite, were higher in humans, 30 to 35.0 μg/ml, than in swine, 12.0 to 20.0 μg/ml. However, swine were able to acetylate sulfisoxazole at a faster rate. The acetyl N^4 metabolite was not separated from dogs' serum.

The distribution constants of sulfisoxazole from the central to the peripheral compartment was greatest in swine, 0.0296 hours^{-1}, and then dogs, 0.0140 hours^{-1}. The distribution from the peripheral to the central compartment was fastest in dogs, 0.0228 hours^{-1}, and then in swine, 0.0162 hours^{-1}. The elimination constants were greatest in swine, 0.5063 hours^{-1}, and then dogs, 0.1565 hours^{-1}. The mean volume of distribution for the central compartment was approximately the same in dogs and swine, 10.60 and 10.48 liters, respectively. The mean volume of the second compartment was larger in swine, 19.76 liters, than in dogs, 6.55 liters.

The fraction of sulfisoxazole bound to serum proteins is capacity limited. At concentrations greater than 200 μg/ml, the fraction bound is reduced from 60% to 40% *in vitro*. This is confirmed by a reduced fraction bound *in vivo* at concentrations greater than 200 μg/ml.

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E. COMPARISON OF THE POTENTIAL TOXICITY OF BILIRUBIN IN HUMANS AND TWO MONOGASTRIC ANIMAL SPECIES AFTER A SINGLE ADMINISTRATION OF SULFISOXAZOLE

ABSTRACT

Administration of sulfonamides during periods of hepato-biliary failure or hepatic immaturity can increase the potential toxicity of unconjugated or indirect bilirubin. The results reported show a small but statistically significant increase of potentially toxic, indirect, or unconjugated bilirubin in dogs after oral administration of sulfisoxazole (100 mg/kg). A similar increase was not observed in swine (100 mg/kg) or humans (approximately 28 mg/kg) after oral or intravenous administration of sulfisoxazole or in humans (approximately 28 mg/kg) after oral administration or in dogs (100 mg/kg) after intravenous administration.

Total and conjugated bilirubin showed small but statistically significant increases in dogs after oral and intravenous administration of sulfisoxazole (100 mg/kg) and in swine after oral administration of sulfisoxazole (100 mg/kg). Total and conjugated bilirubin were significantly correlated in dogs after oral and intravenous administration and in swine after oral administration of sulfisoxazole. The increase in conjugated bilirubin, along with a concomitant increase in total bilirubin, could be due to hepatic induction of glucuronidating capacity or regurgitation of conjugated bilirubin from the hepatocyte instead of excretion into the bile. There was also a significant negative correlation in conjugated and indirect bilirubin, while total bilirubin increased, in dogs after oral and intravenous administration of sulfisoxazole. These data illustrate a difference in species and route of administration when attempting to assess the potential toxicity of bilirubin.

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POULTRY

HEALTH EFFECTS OF SEWAGE SLUDGE AND GRAIN FROM SLUDGE TREATED SOILS VERSUS EQUIVALENT METAL LEVELS IN POULTRY

B. L. Damron, O. Osuna, R. L. Suber, and G. T. Edds

ABSTRACT

Duplicate experiments of 21 days duration were conducted with day-old broiler-type chicks to study the influence of replacing one-half or all of the normal dietary corn complement with corn grown on soil fertilized with liquid Pensacola sludge (LPS).

Neither level of sludge corn had any adverse effect upon final body weights or daily feed intake. The feed conversion values of experiment 1 were not significantly influenced by treatment; however, a statistically significant decrease of efficiency was noted for the all-sludge corn treatment of experiment 2.

In 2 studies with laying hens, the partial or total substitution (50 or 100%) of sludge fertilized corn for that produced with commercial fertilizer had no statistically significant effects upon any of the production parameters measured in experiment 1. In experiment 2, the 100% sludge corn treatment was associated with a significantly increased daily feed intake and final body weight. Hatchability parameters and taste panel results for eggs indicated no significant relationship to dietary treatment. Mineral assays of blood samples and liver, kidney, and muscle tissues from hens and broilers were not influenced by dietary treatment.

Levels of 0, 3, or 6% dried Chicago sludge (DCS) were substituted into the diet of broiler chicks while equivalent nutrient levels were maintained. In addition, 4 other treatments in experiment 1 and 5 in experiment 2 contained the amounts of cadmium, chromium, copper, and iron from reagent sources equivalent to the levels of these elements coming from 6% DCS.

In experiment 1, the feeding of iron or chromium resulted in significant body weight depressions. Only the feed intake of the birds receiving the iron treatment was significantly below that of the control group. Both iron levels in experiment 2 (2,993 mg/kg and 2,196 mg/kg) depressed body weights and daily feed intake. The cadmium and iron treatments of both studies resulted in elevated liver and kidney levels of these minerals. There was also a trend of increasing cadmium residues in the liver and kidney resulting from increasing DCS levels; however, the utilization rate from DCS appeared to be only approximately 20%.

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Levels of 3.5 and 7% DCS sludge were fed to hens in 2 experiments. In addition, amounts of cadmium, chromium, or copper equivalent to those found in the 7% DCS diet were fed from reagent sources.

In the first experiment, none of the production criteria were significantly influenced. In experiment 2, the addition of iron resulted in a numerical depression of egg production. Daily feed intake was significantly reduced by the iròn level fed. Hatchability data was not found to be consistently influenced by dietary treatment in either experiment.

INTRODUCTION

Activated sludge has been the subject of animal feeding trials for well over 20 years. The material is a concentrated source of nitrogen for the ruminant animals, and, even though the biological value of its protein has been determined to be in the neighborhood of 50%; research with ruminants has indicated that the nitrogen retention from activated sludge is equal to that from soybean oil meal or urea (Hurwitz, 1957). Hurwitz (1957) and Schendel and Johnson (1954) found that a level of 2% sludge provided a satisfactory source of vitamin B_{12} for growth. Levels up to 3% gave an additional response that could not be attributed to the presence of vitamin B_{12} alone and was felt to indicate the action of unknown growth factors; possibly due to the fermentation process involved. Firth and Johnson (1955) found that dry activated sludge could be included in the diet of the baby pig up to a level of 5% without adversely affecting growth. In their chick trials, levels of 2 and 10% in the diet produced a growth response in excess of 8% over the control group. Scott and Adams (1955) found that as little as 1% sludge depressed growth when the diet contained 100 units of vitamin D, but that 4% was tolerated very well when 200 units of vitamin D were present.

The technique of municipal sewage processing has also been applied to the treatment of citrus waste from commerical processing plants in Florida (McNary *et al.*, 1953). Many commerical plants are presently using these procedures for the handling of plant waste water. Recently, Damron *et al.* (1975) have evaluated the use of activated citrus sludge in poultry feeds and found it to be an acceptable ingredient at levels between 5 and 7.5% of the diet. No significant differences were found in carcass weight, percent cooking loss, shear force, or sensory evaluation of broiler meat. The current trials were designed to measure the effects upon poultry (chicks and hens) of feeding corn that had been fertilized with DPS or the feeding of low levels of sludge (DCS) itself.

CHICKS RECEIVING DIETS CONTAINING CORN FERTILIZED WITH MUNICIPAL SLUDGE

EXPERIMENTAL PROCEDURE

Duplicate experiments were conducted with day-old broiler-type chicks (Cobb) housed in Petersime battery brooders with raised wire floors to study the influence of replacing one-half or all of the normal dietary

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corn amount with corn grown on LPS treated soil. Table 1 presents the composition of the 3 diets employed in these experiments.

The corn (Pioneer hybrid '3369A') was grown by Dr. M. C. Lutrick, soil chemist, I.F.A.S. Agricultural Research Center, Jay, Florida on plots from an experiment that was begun in 1974 to determine the effects of LPS on the growth and yield. The sludge contained 2.5 to 3.5% solids and was spread on the plots prior to planting and disked into the soil. The sludge plots hadreceived 22.5 cm of sludge in 1976 (the year of test corn harvest) and a total of 60 cm during the 3-year period. The control plots received an annual application of 500 kg/ha of 0-10-20 commerical fertilizer at planting and 250 kg/ha of ammonium nitrate as a sidedress application.

TABLE 1. COMPOSITION OF EXPERIMENTAL DIETS

Ingredients	Control	50% Sludge Corn	100% Sludge Corn
Yellow corn	62.27	31.14	--
Sludge corn	--	31.14	62.27
Soybean meal (48.5%)	31.00	31.00	31.00
Alfalfa Meal (20%)	2.50	2.50	2.50
Ground limestone	1.36	1.36	1.36
Dicalcium Phosphate	1.94	1.94	1.94
Iodized salt	0.35	0.35	0.35
Micro-ingredient mix[1]	0.50	0.50	0.50
DL-Methionine	0.08	0.08	0.08

[1] Supplied the following activities per kilogram of diet: Vitamin A, 6600 I.U.; vitamin D_3, 2200 I.C.U.; menadione dimethylpyrimidinol bisulfite, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid, 13.2 mg; niacin, 39.6 mg; choline chloride, 499.4 mg; vitamin B_{12}, 22 mcg; ethoxyquin, 0.0125%; manganese, 60 mg; iron, 50 mg; copper, 6 mg; cobalt, 0.0198 mg; iodine, 1.1 mg; zinc, 35 mg.

Five male and 5 female chicks were assigned to each of 6 replicate pens in each experiment. Feed and water were provided *ad libitum* and lighting was continuous. At the end of the 21 day feeding period, birds were individually weighed, total feed consumption measured for each pen, and values for average body weight, daily feed intake, and feed per unit of body weight calculated. Mortality was recorded on a daily basis. Performance data were analyzed with analyses of variance and Duncan's multiple range test procedures using the Statistical Analysis System outlined by Barr *et al.* (1976). Representative samples of kidney, liver, and muscle tissue were collected from 4 pens which received each dietary treatment of experiment 2. Two male and 2 female samples were taken from each pen for mineral analysis.

RESULTS AND DISCUSSION

The summary of data outlined in Table 2 indicates that the use of either level of sludge corn had no adverse effect upon final body weights or daily feed intake.

TABLE 2. PERFORMANCE DATA FOR BROILER CHICKS FED CORN FERTILIZED WITH MUNICIPAL SLUDGE
(Experiment 1 and 2)

	Final Body Weight		Daily feed, g		Feed/body weight, g	
Treatment	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2[1]
Control	495	509	34.9	38.6	1.56	1.60a
50% Sludge Corn	495	525	35.1	39.9	1.53	1.56a
100% Sludge Corn	501	499	35.5	40.1	1.53	1.72b

[1] Means without common letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

Feed conversion, in terms of feed required per unit of body weight was not affected in experiment 1, but a significant decrease in efficiency was associated with the 100% sludge corn treatment of experiment 2. This depression was probably due to the degeneration of corn quality which had occurred because of prolonged storage time. Mortality was not found to be treated related, since the 3 birds lost were from control pens.

Analyses of liver, kidney, and muscle tissues for a range of mineral elements provided no clear-cut trends of accumulation due to sludge fertilized corn feeding (A-PY-1; A-PY-2).

HENS RECEIVING DIETS CONTAINING CORN FERTILIZED WITH MUNICIPAL SLUDGE

EXPERIMENTAL PROCEDURE

Two experiments were initiated with White Leghorn hens in approximately 6 months of production to study the effects of feeding various levels of yellow corn (0, 50, and 100% of the dietary corn complement) fertilized with LPS upon egg production and quality parameters. The initial experiment was of 84 days duration while the second was extended to 112 days. The composition of the 3 diets fed in which one-half or all of the control corn was replaced with sludge fertilized corn are summarized in Table 3. Both control and sludge treated corn samples were produced according to procedures outlined in the previous sections concerning chick feeding experiments. In each experiment, 8 replicate groups of 5 individually caged hens were assigned to each of 3 dietary treatments.

Records of egg production and mortality were maintained on a daily basis. Data concerning feed efficiency and daily feed intake were summarized and calculated, along with average egg production at the end of each 28-day interval. A 3-day collection of eggs from each pen was used at the end of each production period for the determination of average egg weights, Haugh unit (interior egg quality) and specific gravity (egg shell quality) scores.

TABLE 3. COMPOSITION OF DIETS CONTAINING YELLOW CORN FERTILIZED WITH MUNICIPAL SLUDGE (EXP. 1 AND 2)

Ingredient	Control	50% Sludge Corn	100% Sludge Corn
Control Corn	66.97	33.48	--
Sludge Corn	--	33.48	66.97
Soybean Meal (48.5%)	20.0	20.0	20.0
Alfalfa Meal (20%)	2.5	2.5	2.5
Animal Fat	.50	.50	.50
Ground Limestone	6.88	6.88	6.88
Dicalcium Phosphate (18.5%, 24% Ca)	2.25	2.25	2.25
Iodized Salt	.35	.35	.35
Microingredient mix[1]	.50	.50	.50
DL-Methionine	.05	.05	.05

[1] Supplied the following activities per kilogram of diet: Vitamin A, 6600 I.U.; vitamin D_3, 2200 I.C.U.; menadione dimethylpyrimidinol bisulfite, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid, 13.2 mg; niacin, 39.6 mg; choline chloride, 499.4 mg; vitamin B_{12}, 22 mcg; ethoxyquin, 0.0125%; manganese, 60 mg; iron, 50 mg; copper, 6 mg; cobalt, 0.0198 mg; iodine, 1.1 mg; zinc, 35 mg

Hens were artificially inseminated with pooled semen at the end of the second and third experimental periods in experiment 1, and third and 4th periods in experiment 2. A collection of 7 days egg production was placed in the incubator each time for determination of fertility, fertile hatchability, and total hatchability. All of the above data were subjected to the analysis of variance and Duncan's multiple range test procedures cited previously in the section concerning chick feeding trials.

In experiment 1, 2 small triangular taste panel trials were performed to determine if sludge fertilized corn imparted any objectionable flavor to eggs. Panelists were asked to select the one sample that was different from among the 3 presented. Also, blood samples were taken from experiment 1 hens at 5 intervals during the experiment in an attempt to monitor any change in the mineral status of the hen as feeding progressed. At termination, 10 representative hens from each treatment of experiment 1, and 12 hens from each treatment of experiment 2 were sacrificed for mineral analyses of liver, kidney, and muscle tissue.

RESULTS AND DISCUSSION

The performance data of Table 4 for the hens of experiment 1 indicate that the partial or total substitution of LPS fertilized corn for that produced with commercial fertilizer had no statistically significant effects upon any of the production parameters measured.

TABLE 4. PERFORMANCE DATA OF WHITE LEGHORN HENS FED VARIOUS LEVELS OF CORN FERTILIZED WITH LPS (EXP. 1)

Treatment	Production	Feed/doz[1] kg	Daily feed[1] g	Egg wts g	Specific Gravity	Haugh Units	Final Body Wt, g
Control	70.78	1.68	96.3	62.9	1.078	72.2	1544
50% Sludge Corn	70.59	1.72	96.6	61.6	1.077	71.6	1686
100% Sludge Corn	68.85	1.80	102.6	62.8	1.078	70.6	1773

[1] Average of first 2 production periods. The third period was lost due to a weighing error.

Increased daily feed intake and feed efficiency values noted for the all sludge corn diet were probably the result of corn quality deterioration during storage. It should be noted that this dietary treatment was associated with the highest body weights of the study, indicating that some of the extra feed went towards fat production.

Very similar trends to those outlined above are present in the results of experiment 2 (Table 5).

Egg production, feed efficiency, egg weight, specific gravity, and Haugh units were not significantly affected by the presence of sludge fertilized corn in the diet. The increased feed intake for the 100% replacement group was significantly different from the other treatment and control. Body weight was significantly higher for the 100% treatment.

TABLE 5. PERFORMANCE DATA OF WHITE LEGHORN HENS FED VARIOUS LEVELS OF CORN FERTILIZED WITH MUNICIPAL SLUDGE (EXP. 2)

Treatment	% Hen-Day egg Production	Feed/doz kg	Daily Feed g	Egg Wt	Specific Gravity	Haugh Units	Final Body Wt, g
Control	68.4	1.95	107.9a	67.2	1.082	74.0	1697a
50% Sludge Corn	65.8	1.95	105.2a	66.1	1.081	71.8	1662a
100% Sludge Corn	68.7	1.95	113.1b	66.9	1.080	72.1	1796b

[1] Means without common letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

In both experiments, hatchability parameters were low for all groups and not significantly affected by dietary treatment (Tables 6 and 7). Taste panel results (Table 8) indicated that no detectable off-flavors were imparted to eggs by the feeding of sludge fertilized corn. Participants were not able to successfully select the egg sample that differed from the remaining 2 identical ones presented.

Mineral levels from blood samples taken before treatment and at 4 intervals during the first experiment were extremely variable and provided no indication that blood mineral levels were elevated due to the inclusion of sludge fertilized corn in the diet (A-PY-3). Results from similar assays on liver, kidney, and muscle samples at the end of each experiment also displayed inconsistent variation and revealed no trends indicating a treatment effect (A-PY-4; A-PY-5).

TABLE 6. HATCHABILITY DATA OF WHITE LEGHORN HENS FED VARIOUS LEVELS OF CORN FERTILIZED WITH LPS (EXP. 1)

Treatment	% Fertility		% Fertile Hatch		% Total Hatch	
	Hatch 1	Hatch 2	Hatch 1	Hatch 2	Hatch 1	Hatch 2
Control	67.4	71.3	94.1	91.5	63.3	64.7
50% Sludge Corn	67.4	77.7	86.0	88.7	58.2	68.9
100% Sludge Corn	57.5	64.4	87.0	91.4	50.1	58.8

TABLE 7. HATCHABILITY DATA OF WHITE LEGHORN HENS FED VARIOUS LEVELS OF CORN FERTILIZED WITH LPS (EXP. 2)

Treatment	% Fertility		% Fertile Hatch		% Total Hatch	
	Hatch 1	Hatch 2	Hatch 1	Hatch 2	Hatch 1	Hatch 2
Control	78.0	42.9	83.6	60.5	68.2	41.7
50% Sludge Corn	70.8	47.6	72.2	69.3	60.3	40.2
100% Sludge Corn	79.7	49.5	80.2	67.9	61.9	41.6

TABLE 8. TASTE PANEL RESULTS FOR EGGS FROM HENS FED VARIOUS LEVELS OF CORN FERTILIZED WITH LPS (EXP. 1)

Treatment	Correct responses/number of panelists		
	Trial[1]	Trial[2]	Combined[1]
50% Sludge Corn	4/10	3/10	7/20
100% Sludge Corn	4/10	4/10	8/20

[1] Significance ($P < 0.05$) = 7/10 or 14/20

CHICKS FED VARIOUS LEVELS OF CHICAGO SLUDGE AND MINERALS

EXPERIMENTAL PROCEDURE

In each of 2 experiments, 8 day old Cobb color-sexed broiler chicks (4 males and 4 females) were randomly assigned to each pen of a heated Petersime battery brooder with raised wire floor. Four replicate pens were assigned to each of 7 dietary treatments for a 3-week feeding period. Levels of 0, 3, or 6% DCS were substituted into the basal diet with attention to maintaining equivalent nutrient levels (metabolizable energy, crude protein, calcium, phosphorus, sulfur amino acids, and lysine) across the 3 diets (Table 9). Six percent of the material was a necessary upper limit because this was the greatest amount that could be included in the formulation when sludge was assumed to have no nutritional value. In addition, 4 other treatments in experiment 1, and 5 in experiment 2 contained the amounts of cadmium, chromium, copper, and iron from reagent sources equivalent to the levels of these elements coming from 6% sludge.

TABLE 9. COMPOSITION OF DIETS (%)

Ingredients	Control	3% Sludge	6% Sludge
Yellow Corn	48.00	48.00	48.00
Soybean Meal (48.5%)	35.30	35.30	35.30
Defluorinated phosphate (18% P and 32% Ca)	1.91	1.91	1.91
Limestone	1.26	1.26	1.26
Iodized salt	.35	.35	.35
Microingredients[1]	.50	.50	.50
Animal fat	6.52	6.52	6.52
DL-Methionine	.16	.16	.16
Dried Chicago Sludge	--	3.00	6.00
Sand	6.00	3.00	--

[1] Ingredients supplied per kilogram of diet: Vitamin A, 6600 IU, vitamin D_3, 2200 ICU; menadione dimethyl-pyrimidinol bisulfite, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid, 13.2 mg; niacin, 39.6 mg; choline chloride, 499.4 mg; vitamin B_{12}, 22 mcg; ethoxyquin, 0.0125%; manganese, 60 mg; iron, 50 mg; copper, 6 mg; cobalt, 0.0198 mg; zinc, 35 mg.

Table 10 indicates the source and amount of each element added. The additional iron level fed in experiment 2 resulted from a second analysis of the sludge where a somewhat lower iron level was reported. Standard parameters of body weight, feed intake, feed efficiency (g feed/g final body weight) and mortality were evaluated along with general observation of the condition of the birds. Samples of liver, kidney, and muscle were taken in each experiment from 2 birds of each sex from 3 replicate pens of each treatment.

TABLE 10. LEVELS OF ELEMENTS ADDED TO CHICK DIETS[1]

Chromium from chromic sulfate	150 mg/kg
Cadmium from cadmium chloride	12 mg/kg
Copper from cupric sulfate	92 mg/kg
Iron from ferrous sulfate	2,992 mg/kg
(Exp. 2)	2,196 mg/kg

[1] Levels determined from laboratory analysis of DCS x 6%.

RESULTS AND DISCUSSION

Three-week performance data presented in Table 11 reflect a clearly significant depression of body weight from the supplemental inclusion of 2,992 mg/kg iron in the feed. This value was also significantly lower than any other treatment weight.

TABLE 11. PERFORMANCE OF BROILER CHICKS FED VARIOUS LEVELS OF DCS OR MINERALS (EXP. 1)[1]

Diets	Body Weight (g)	Feed Intake (g)	Feed Efficiency (g intake / g BW)
Control	560^{a}	38.5^{ab}	1.43^{b}
+ 3% DCS	541^{ab}	38.1^{ab}	1.48^{b}
+ 6% DCS	545^{ab}	38.2^{ab}	1.47^{b}
+ 2,992 mg/kg Fe	450^{c}	34.4^{c}	1.63^{a}
+ 12 mg/kg Cd	540^{ab}	39.2^{a}	1.52^{b}
+ 92 mg/kg Cu	552^{b}	38.8^{a}	1.47^{b}
+ 150 mg/kg Cr	525	36.3^{bc}	1.45^{b}

[1] Means without common letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

The dietary addition of chromium was associated with a significantly depressed body weight. However, the level employed (150 mg/kg) was below that found toxic (300 mg/kg) by Kunishisa *et al.* (1966).

Feed intake values of experiment 1 were reduced statistically only for the group receiving iron. It is very difficult to determine if this is the cause of effect of the depressive results seen in body weights. Feed efficiency is a calculation involving the other variables previously discussed; therefore, only iron significantly reduced this parameter.

Again, in the second experiment (Table 12) iron was the only element to depress body weights.

TABLE 12. PERFORMANCE OF BROILER CHICKS FED VARIOUS LEVELS LEVELS OF DCS OR MINERALS (EXP. 2)[1]

Diets	Body Weight (g)	Feed Intake (g)	Feed Efficiency (g intake/g BW)
Control	576^{a}	41.4^{a}	1.50^{d}
+ 3% DCS	563^{a}	40.8^{ab}	1.53^{bcd}
+ 6% DCS	553^{a}	40.6^{ab}	1.54^{bcd}
+ 150 mg/kg Cr	559^{a}	41.7^{a}	1.56^{abcd}
+ 12 mg/kg Cd	528^{a}	41.9^{a}	1.68^{a}
+ 91.5 mg/kg Cu	559^{a}	40.6^{ab}	1.52^{cd}
+ 2,992 mg/kg Fe	439^{b}	35.1^{c}	1.66^{ab}
+ 2,196 mg/kg Fe	470^{b}	36.9^{bc}	1.65^{abc}

[1] Means without common letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

The degree of weight reduction did appear to be correlated with the amount of iron fed. These results are somewhat in contrast to those of Deobald and Elvehjem (1935) where the toxic level was identified as approximately 4,500 mg/kg. Feed intake was reduced, as in experiment 1, by the presence of either iron level. Feed conversion values for both iron and cadmium treatments were also significantly poorer than that of the control group. Although cadmium did not cause significant body weight depressions, a numerical reduction can be noted in both experiments. It would appear that these birds were borderline to a toxic situation even though the National Research Council (1977) indicates 20 mg/kg to be toxic. Mortality was not a factor in either experiment.

Tissue data of both experiments (A-PY-6 to A-PY-9) indicated no accumulation of minerals was fostered in muscle tissue by the feeding of DCS or supplemental elements. The cadmium and iron treatments of both studies resulted in elevated liver and kidney levels of those minerals in proportion to their supplementation. There was also a trend of increasing cadmium residues in liver and kidney resulting from increasing sludge levels; however, the utilization rate from sludge appeared to be only approximately 20%. The liver appeared to be the greatest accumulator of iron, with increases related to sludge addition occurring in the first experiment but not the second. The response of kidney tissue to iron supplementation was definite. Copper values were not altered by any of the dietary variables and chromium in tissues was below detection limits.

HENS FED VARIOUS LEVELS OF CHICAGO SLUDGE AND MINERALS

Eight replicate pens of 5 individually caged White Leghorn hens were assigned to each of 6 dietary treatments for an 84-day feeding period in experiment 1, and 112 days for the second trial. Levels of 3.5 and 7% DCS were substituted into a basal diet with attention to maintenance of equivalent nutrient levels across the 3 diets (Table 13).

TABLE 13. COMPOSITION OF BASAL HEN DIET FOR SLUDGE ADDITIONS (EXPERIMENTS 1 AND 2)

Ingredient	Percentage
Yellow Corn	58.29
Soybean Meal (48.6%)	21.35
Animal Fat	4.00
Limestone	6.55
Dicalcium Phosphate	1.90
DL-Methionine	.06
Iodized Salt	.35
Microingredient mix[1]	.50
Inert Filler	7.00

[1] Ingredients supplied per kilogram of diet: Vitamin A, 6600 IU; vitamin D$_3$, 2200 ICU; menadione dimethyl-pyrimidinol bisulfite, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid, 13.2 mg; niacin, 39.6 mg; chloride, 499.4 mg; vitamin B$_{12}$, 22 mcg; ethoxyquin, 0.0125%, manganese, 60 mg; iron, 50 mg; copper, 6 mg; cobalt, 0.0198 mg; zinc, 35 mg.

In addition, 3 other treatments in both studies include the provision of amounts of cadmium, chromium, or copper from reagent sources equivalent to those found in the 7% diet. Those reagent sources were chromic sulfate, copper sulfate, and cadmium chloride in both experiments. Since iron had been found to cause reduced body weights in chick experiments, it was also included in the second study from ferrous sulfate.

Records of egg production, egg weight, daily feed intake, feed efficiency, specific gravity, Haugh units, and mortality were summarized or calculated at 28-day intervals. Also, fertility and hatchability was determined by artificial insemination during the second and third periods of experiment 1, and the third and fourth months of experiment 2. Birds were individually weighed at the time of termination. Samples of eggs, feed, kidney, muscle, liver, and feces were taken from each treatment group for mineral analyses.

RESULTS AND DISCUSSION

In experiment 1, none of the production criteria shown in Table 14 (egg production, daily feed intake, feed efficiency, egg weights, and final body weights) were statistically affected by the addition of up to 7% DCS to the diet or the feeding of comparable mineral levels from reagent sources. Specific gravity (egg shell quality) was significantly improved in eggs from hens that received 7% DCS. Although interior egg quality (Haugh units) was significantly reduced when compared to the value associated with 7% DCS, it was not different from the control value. This author feels that biological variation was largely responsible for this effect.

TABLE 14. PERFORMANCE DATA OF WHITE LEGHORN HENS FED VARIOUS LEVELS OF DCS OR MINERALS (EXP. 1)

Treatment	% Hen-Day egg Production	Feed/ doz (kg)	Daily Feed (g)	Egg Wt (g)	Specific Gravity[1]	Haugh Units[1]	Final Body Wt (g)
Control	76.03	1.56	99.2	62.1	1.0839^{bc}	73.4^{ab}	1634
+ 3.5% DCS	65.81	1.56	98.5	61.2	1.0846^{abc}	74.3^{b}	1634
+ 7.0% DCS	72.56	1.62	97.4	60.7	1.0861^{a}	74.6^{b}	1654
+ 14 mg/kg Cd	73.63	1.56	95.7	61.5	1.0828^{c}	74.1^{b}	1527
+ 106.8 mg/kg Cu	73.85	1.60	98.5	60.3	1.0856^{ab}	71.9^{a}	1617
+ 175 mg/kg Cr	72.97	1.60	100.4	61.5	1.0844^{abc}	73.6^{ab}	1705

[1] Means without common letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

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In experiment 2 (Table 15), the addition of iron from a reagent source produced the main significant effect. The egg production of birds receiving iron did not differ statistically from that of controls, but was numerically depressed, and significantly lower than all but the chromium treatment group. A similar reaction was noted in final body weights where the iron treatment differed from all but the control and cadmium groups.

Daily feed intake was significantly reduced for the birds whose diet was supplemented with iron, while Haugh unit scores of eggs from all treatments were numerically or statistically better than controls. Egg weight and specific gravity data were not found to be significantly influenced by treatment.

TABLE 15. PERFORMANCE DATA OF WHITE LEGHORN HENS FED VARIOUS LEVELS OF DCS AND MINERALS (EXP. 2)

Treatment	% Hen-Day egg Production	Feed/ doz (kg)	Daily Feed (g)	Egg Wts (g)	Specific Gravity[1]	Haugh Units	Final Body Wt (g)[1]
Control	74.6^{ab}	1.592^{abc}	95.7^{a}	63.01	1.077	76.25^{bc}	1627^{ab}
+ 3.5% DCS	75.79^{a}	1.553^{c}	96.02^{a}	62.08	1.078	78.77^{abc}	1709^{a}
+ 7.0% DCS	77.3^{a}	1.516^{c}	96.2^{a}	61.24	1.076	81.30^{a}	1681^{a}
+ 14 mg/kg Cd	77.27^{a}	1.525^{c}	96.65^{a}	62.0	1.077	80.04^{ab}	1576^{b}
+ 106.8 mg/kg Cu	75.96^{a}	1.569^{bc}	97.17^{a}	62.7	1.076	78.02^{bc}	1718^{a}
+ 175 mg/kg Cr	73.42^{ab}	1.645^{ab}	96.28^{a}	63.08	1.077	79.58^{ab}	1678^{a}
+ 2,562 mg/kg Fe	66.83^{b}	1.678^{a}	89.65^{b}	62.42	1.077	79.95^{ab}	1538^{b}

[1] Means without common letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

Hatchability data was not found to be consistently influenced by dietary treatment in either experiment (Tables 16 and 17). In experiment 1 (Table 16), the values approximated those seen in the industry and did not appear to be treatment related. This is illustrated in the fertile and total hatchability areas, where control groups were generally the lowest of any dietary regime. The figures presented in Table 17 for the second experiment were extremely variable and, although some depressions are exhibited for the 7% sludge and copper treatments, they were not consistent over the 2 hatches and were not felt to represent a true treatment effect.

TABLE 16. HATCHABILITY DATA OF WHITE LEGHORN HENS FED VARIOUS LEVELS OF DCS OR MINERALS (EXP. 1)

	% Fertility		% Fertile Hatch		% Total Hatch	
Treatment	Hatch 1	Hatch 2	Hatch 1	Hatch 2	Hatch 1	Hatch 2
Control	89.4	80.2	79.5	88.0	67.2	71.0
+ 3.5% DCS	89.1	76.5	80.9	86.7	72.6	71.2
+ 7.0% DCS	94.2	86.9	82.7	93.0	82.2	82.0
+ 14 mg/kg Cd	90.1	77.1	79.9	91.5	75.3	72.1
+ 106.8 mg/kg Cu	94.9	85.0	86.3	94.9	84.0	83.7
+ 175 mg/kg Cr	90.8	88.7	87.4	91.7	77.3	81.4

TABLE 17. HATCHABILITY DATA OF WHITE LEGHORN HENS FED VARIOUS LEVELS OF DCS OR MINERALS (EXP. 2)

	% Fertility		% Fertile Hatch		% Total Hatch	
Treatment	Hatch 1	Hatch 2	Hatch 1	Hatch 2	Hatch 1	Hatch 2
Control	76.4	68.4	85.5	93.1	66.3	63.7
+ 3.5% DCS	75.4	74.1	94.0	83.6	70.5	62.1
+ 7.0% DCS	88.0	82.2	81.4	52.0	71.6	42.5
+ 14 mg/kg Cd	74.7	82.7	87.9	83.2	66.0	68.6
+ 106.8 mg/kg Cu	55.9	72.8	92.6	85.3	53.4	61.6
+ 175 mg/kg Cr	79.5	79.9	91.1	83.3	72.5	66.2
+ 2,562 mg/kg Fe	76.4	82.3	82.5	89.0	63.5	74.2

Table 18 summarizes the analytical data taken on the feeds of the first experiment where it can definitely be seen that the addition of sludge did contribute significant amounts of the mineral elements under study to the diets.

Table 19 present the same type of data for fecal samples, indicating that large amounts of these elements were being passed through unabsorbed in proportion to the feed's content. It is felt that this is what happens to the majority of the minerals received by the chickens through sludge additions to the feed.

TABLE 18. ANALYSES OF FEEDS CONTAINING DCS OR MINERALS (EXP. 1)

Treatment	Cu	Fe	Al	Cd	Cr
Control	10	188	275	0	3
+ 3.5% DCS	50	725	550	2.8	100
+ 7.0% DCS	73	1525	550	1.8	163
+ 14 mg/kg Cd	10	250	325	8.1	6
+ 106.8 mg/kg Cu	93	225	175	0	1
+ 175 mg/kg Cr	10	213	250	0	125

TABLE 19. FECAL ANALYSES FROM WHITE LEGHORN HENS FED VARIOUS LEVELS OF DCS OR MINERALS (EXP. 1)

Treatment	Cu	Fe	Al	Cd	Cr
Control	28	750	1176	.62	9
+ 3.5% DCS	183	3534	2312	.22	265
+ 7.0% DCS	414	7125	3656	.66	656
+ 14 mg/kg Cd	31.5	663	694	.58	9.5
+ 106.8 mg/kg Cu	144.5	888	944	.69	9
+ 175 mg/kg Cr	30.5	1088	1076	.62	519

Analyses of eggs from the hens of experiment 1 did not indicate any effect from any of the dietary additions (A-PY-10). In contrast, the tissue analyses results from experiment 1 (A-PY-11) did point out that increased mineral stores would result from sludge and reagent mineral feeding. In liver, both cadmium and iron were increased stepwise as the sludge content increased. Cadmium was at its highest level when fed from the purified source, indicating a difference in availability from the 2 materials. Liver copper was not altered by treatment and chromium was within detection limits only for the supplemental chromium treatment. Similar trends are present in the data concerning kidney tissue. Muscle minerals did not seem to be influenced to any appreciable exten by any of the dietary additions.

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HEALTH EFFECTS IN MICE FED DIETS THAT CONTAINED TISSUES FROM CATTLE OR SWINE FED SEWAGE SLUDGE RATIONS

K. C. Kelley, R. L. Shirley, O. Osuna, J. Bertrand, and G. T. Edds

ABSTRACT

At the end of the swine and cattle feeding periods (Bertrand *et al.*, 1979; Edds *et al.*, 1979) the animals were slaughtered and kidney and liver samples collected and frozen. The frozen tissues were sliced and ground in a Hobart grinder and freeze-dried. The tissue samples were powdered by grinding and their protein content determined by Kjeldahl technique (Official Methods of Analysis, AOAC Eleventh Edition, 1975, William Horwitz, Editor).

Swiss mice, 45 days of age, were fed freeze-dried liver and kidney from cattle or swine fed a control diet, sewage sludge, corn grain, or sorghum forage from land covered with liquid sewage sludge. Mice fed diets containing 5% cattle kidney had higher levels of Cu, Fe, Co, and Pb in their kidney than mice fed 10% cattle liver in diets. Nickel was higher in kidney, liver, and muscle; Fe, Co, and Pb higher in liver, Cu, Co, and Pb higher and Zn lower in muscle; and Cr higher in kidney of mice fed diets that contained 5% kidney from cattle. F_1 females had a 6-fold increase in liver Cd in the DCS treatment, while F_2 females showed no differences in tissue minerals due to DCS treatments. F_1 females fed liver had a decrease in number born in both sludge treatments.

F_0 mice fed kidney and liver from cattle fed sorghum that received Pensacola liquid sludge (LPS) showed no changes in minerals in kidney, liver, and muscle due to treatment or diet. Number born per litter was greater in F_0 mice fed kidney in the LPS treatment but there was no effect when liver was fed.

Liver from swine F_0 fed 0, 10, or 20% University of Florida digested sewage sludge (DFS) in diets of mice showed an increase in the Pb content of mice liver and muscle at the 20% DFS level.

Mice fed liver from swine F_{1-2} in diets had higher levels of Cd in liver, kidney, and muscle tissue in the 10% DFS group. Pb in mice liver was increased in the 10% DFS and in muscle in both the 10 and 20% DFS groups. Mice fed kidney from swine F_{1-3} had high levels of Cd in liver.

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INTRODUCTION

All mice utilized in the feeding trials were housed in stainless steel cages with wire mesh bottoms.[1] Water was supplied *ad libitum*. Diets were fed in glass jar feeders with stainless steel tops.[2] Diets were supplied *ad libitum* with the feeders being changed each two or three days as needed. Littering cages were stainless steel with solid bottoms and Sanicel[3] was used as the contact bedding.

At the termination of the feeding trial, each mouse was sacrificed by cervical separation, and body, liver, and kidney weights determined. Liver, kidney, and muscle samples were taken from each mouse. Within each treatment, the tissue samples were pooled and selected mineral analyses of kidney, liver, and muscle were done by atomic absorption.[4]

Feeding Trial Designs

FT #1 - Cattle kidney and liver tissue--Thirty-two Cox (Swiss) outbred mice[5] (16 male, 16 female), 25-45 days of age, were assigned to each treatment group. Mice of the same sex were housed two per cage. After 15 days on the diets, one male of the same treatment was introduced into each cage of females for breeding purposes. Twenty days later, females were put in littering cages. The number of young born and the date were recorded. At 21 days of age, the F_1 offspring were counted, 16 females per treatment were removed for continuation on the diets; the remainder of the F_1 were sacrificed along with the 32 F_0 mice for tissue samples. The mice sacrificed were between day 60 and day 70 of the feeding trial period. The F_1 females fed 5% kidney in their diet were continued an additional 60 days and sacrificed. The F_1 females fed 10% liver in the diet had normal adult males introduced for breeding on day 160. On day 180, females were placed in solid bottom litter cages (two per cage) and the date of littering of the F_2 generation and the number per litter recorded. The F_2 generation at 21 days of age were counted and sacrificed along with the F_1 parent female. Kidney, liver, and muscle tissue samples were taken on all mice at the end of the feeding trial for mineral assays.

FT #2 - Swine liver tissue--Forty Cox (Swiss) outbred mice[5] (20 male and 20 female), 35-45 days of age, were assigned to each treatment group. Five mice of the same sex were housed per cage. Freeze-dried liver from swine F_0 treatments were fed for a 100 day trial and freeze-dried liver from swine F_{1-2} treatments were fed for a 34 day trial. At the end of the feeding trial period, all mice were sacrificed and tissue samples taken. Because of limited kidney tissue availability, this trial was performed using liver tissue only.

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[1] LC-75/SA and LC-75/SB cages. Wahmann Manufacturing Co., Timonium, MD

[2] LC-207/A Mouse Feeder, Wahmann Manufacturing Co., Timonium, MD

[3] Paxton Processing Co., Inc., Univarium Research Inc., White House Station, NJ

[4] Atomic absorption analyses performed by Soils Department, University of Florida, Gainesville, FL

[5] Laboratory Supply Co., Inc., Indianapolis, IN

FT #3 - Swine kidney and liver tissue--Twelve female Cox (Swiss) outbred mice[1], 45 days of age, were assigned to each kidney treatment group. Twenty male and 20 female mice, 35-45 days of age, were assigned to each liver treatment group. Four or five mice of the same sex were housed together. The kidney and liver diets were fed for 15 days. On day 15, normal males were introduced, one per cage to the females on kidney diets. The liver diets had one male of the same treatment introduced into the female cage. On day 35, females were placed in littering cages and the number of young born and the date recorded. At 21 days of age, the F_1 offspring were counted. The F_1 mice and the parent F_0 mice were sacrificed between day 60 and 70 of the feeding trial period.

FT #4 - Cattle kidney and liver tissue--Twelve female Cox (Swiss) outbred mice[1], 45 days of age, were assigned to each kidney treatment group and housed 4 per cage. On day 15, one normal male was introduced into each cage of females for breeding purposes. Thirty-two mice (16 male, 16 female) were assigned to each liver treatment group and housed 4 per cage. On day 15, a male of the same treatment was introduced into each female cage. On day 35, females were placed in littering cages, the number of young born and the date of birth recorded. At 21 days of age, the F_1 offspring were counted; 16 females on each liver diet treatment were removed for continuation on the diet. The remaining F_1 and the F_0 of the liver diet were sacrificed and muscle, kidney, and liver samples taken. All mice on the kidney treatments were sacrificed at 21 days of age of F_1 and tissue samples taken. The F_1 females were continued on their respective liver diets for 180 days. Normal adult males were introduced for breeding on day 240 to 250. On day 260 to 270, females were placed individually in solid bottom litter cages and the date of littering on the F_2 generation and the number in the litter recorded. The F_2 generation at 21 days of age, were counted and sacrificed along with the F_1 parent female. Kidney, liver, and muscle tissue samples were taken from each mouse.

FT #5 - Swine kidney and liver tissue--Twelve female Cox (Swiss) outbred mice[1], 45 days of age, were assigned to each cattle kidney dietary group and 16 female mice were assigned to each liver treatment group. The mice were housed 4 per cage. On day 15, one normal male was introduced into each cage of females for breeding purposes. On day 35, females were placed in litter cages, the number of young born and the date of birth recorded. At 21 days of age, the F_1 offspring were counted. On day 60 of the feeding trial, both the F_0 and F_1 mice were sacrificed, and kidney, liver, and muscle samples were taken for mineral analysis.

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[1] Laboratory Supply Company, Inc., Indianapolis, IN

RESULTS AND DISCUSSION

Data from the feeding trials are presented in Tables 3-9. Feeding trial 1 (Tables 3-9) data on mice fed diets containing freeze-dried kidney and liver tissue of cattle fed dried Chicago digested sludge (DCS) or forage from land covered with Pensacola liquid sludge (LPS) diets are presented. Table 3 gives the final body, liver, and kidney weights as well as liver and kidney to body weight ratios. Mice fed kidney tissue showed changes in the F_0 generation in all parameters but no clear cut pattern emerges between sexes and treatment. There were no differences among treatments in any of the parameters. The F_0 mice fed liver tissue showed differences among treatments and sex in all weight parameters but no consistent pattern occurred. The F_1 female mice fed liver tissue showed no change in their liver/body weight and kidney/body weight ratios, but there were differences among treatments and sex in the parameters of final body weight, liver, and kidney weight.

Mice fed kidney tissue (Table 4) in the 500 g/head/day DCS group or forage groups had no significant differences in litter size in the F_0 generation due to treatment but had a decrease in the number weaned. The F_1 female mice fed kidney-containing diets had no differences among treatment groups in the number weaned. The F_0 mice fed liver tissue had no significant differences in litter size, but the number weaned was affected by sludge treatments. The F_1 females fed liver had a decrease in litter size and in the number weaned at the 500 g/head/day DCS.

Mineral analyses data on tissues of feeding trial #1 are presented in Tables 5-9 with Tables 5 and 6 giving the data of kidney, liver, and muscle tissue of mice fed freeze-dried kidney and liver from cattle fed 500 g/head/day DCS or forage from land receiving 7.6 cm per hectare (cm/ha) of LPS. Mineral analyses data (Table 7) demonstrated higher levels of Cd in kidney and liver tissue of mice that consumed kidney or liver tissue from cattle fed 500 g/head/day DCS. No differences in Cu, Fe, Co, Zn, and Pb were seen in mice kidney and liver tissue. In muscle tissue of mice, there was a significant depression at Cu in both the 7.6 cm/ha LPS and 500 g/head/day DCS treatments. When male and female mice tissue concentrations of the F_0 generation (Table 8) were averaged across feed types and treatment groups, females' kidney tissue had a higher concentration of Cd than the males. Male liver tissue had a higher concentration of Cu than that of females. Muscle tissue of mice showed no differences between the sexes for any of the minerals. When mice datawere pooled across treatments and sex (Table 9), diets containing 5% cattle kidney and 10% cattle liver were compared. Mice kidney tissue was found to contain significantly higher concentrations of Cu, Fe, Co, and Pb when the mice consumed 5% cattle kidney in their diet. The mice liver tissue also was found to contain higher levels of Fe, Co, and Pb when 5% kidney was in the diet. The muscle tissue of mice consuming 5% kidney in their diet had significantly higher concentrations of Cu, Co, and Pb than the mice fed 10% liver in the diet; Zn was significantly lower in muscle tissue of mice fed 5% kidney.

TABLE 1. COMPOSITION OF DIETS FED MICE

Ingredient	Percentage	gm/kilo
Protein[1]	15	150
Sucrose	32	320
Corn starch[2]	32	320
Vitamin mix[3]	1	10
Salt mix[4]	4	40
Alphacel[5]	11	110
Corn oil[6]	5	50

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[1] Protein in the diet consists of 5% freeze-dried kidney tissue or 10% freeze-dried liver tissue and Vitamin Free Casein (ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, OH) to yield a total protein of 15%.

[2] Corn starch plus the non-protein portion of the freeze-dried tissue sample.

[2] [3] [4] [5] [6] Ingredient source, ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, OH.

TABLE 2. TREATMENT OF CATTLE AND SWINE CONSUMING SEWAGE SLUDGE SLAUGHTER DATES, AND TISSUES FED MICE IN VARIOUS TRIALS

Feeding Trial	Tissue	Source	Treatments	Date Slaughtered
1	Kidney	Cattle	Control, 7.6 cm/ha LPS[1] or 500 g/head/day, DCS[2]	08/12/77
	Liver	Cattle	Control, 7.6 cm/ha LPS or 500 g/head/day DCS	
2	Kidney	Swine	A. F_0 10% DFS[3] F_0 20% DFS B. F_{1-2} Control F_{1-2} 10% DFS F_{1-2} 20% DFS	04/04/78
	Liver	Swine	A. F_0 Control F_0 10% DFS F_0 10% DFS B. F_{1-2} Control F_{1-2} 10% DFS F_{1-2} 20% DFS	
3	Kidney	Swine	F_{1-3} Control F_{1-3} 10% DFS F_{1-3} 20% DFS	08/12/78
	Liver	Swine	F_{1-3} Control F_{1-3} 10% DFS F_{1-3} 20% DFS	
4	Kidney	Cattle	Control 15.2 cm/ha LPS 22.8 cm/ha LPS	08/12/78
	Liver	Cattle	Control 15.2 cm/ha LPS 22.8 cm/ha LPS	
5	Kidney	Swine	$F_{1-2,1}$ Control $F_{1-2,1}$ 10% DFS $F_{1-2,1}$ 20% DFS	04/19/79
	Liver	Swine	$F_{1-2,1}$ Control $F_{1-2,1}$ 10% DFS $F_{1-2,1}$ 20% DFS	

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[1] Centimeters per hectare, Pensacola liquid sludge (LPS)

[2] Dried Chicago sludge (DCS)

[3] Dried Florida sludge (DFS)

TABLE 3. MICE FEEDING TRIAL 1. BODY AND ORGAN WEIGHTS OF MICE FED DIETS CONTAINING FREEZE-DRIED KIDNEY AND LIVER TISSUE FROM CATTLE FED A CONTROL DIET, CORN FROM LAND RECEIVING 7.6 CM/HA[1] OF LPS[2] OR 500 G/HEAD/DAY DCS[3,4]

Mice parameter	Tissue fed	Generation	Test days	Treatment											
				Control - no sludge				7.6 cm/ha LPS				500 g/head/day DCS			
				n	female	n	male	n	female	n	male	n	female	n	male
Final body wt, g	Kidney	F_0	60	16	32.07 ± 0.99^{a}	11	33.98 ± 1.21^{a}	16	33.20 ± 0.99^{a}	13	38.43 ± 1.12^{b}	16	31.76 ± 0.99^{a}	13	36.36 ± 1.2^{ab}
		F_1	120	11	31.86 ± 0.91			15	32.34 ± 0.78			5	31.39 ± 1.35		
	Liver	F_0	60	16	33.91 ± 0.89^{a}	16	37.36 ± 0.89^{b}	16	36.15 ± 0.89^{a}	15	37.56 ± 0.92^{b}	16	33.33 ± 0.89^{a}	16	34.54 ± 0.89^{c}
		F_1	200	16	42.98 ± 3.53^{a}			16	37.68 ± 3.53^{b}			16	38.76 ± 3.53^{b}		
Liver wt, g	Kidney	F_0	60	16	2.32 ± 1.6^{a}	11	$2.12 \pm .13^{a}$	16	$2.76 \pm .16^{b}$	13	$2.16 \pm .12^{a}$	16	$2.51 \pm .16^{ab}$	13	$2.10 \pm .12^{a}$
		F_1	120	11	$2.32 \pm .11$			15	$2.39 \pm .09$			5	$2.47 \pm .16$		
	Liver	F_0	60	16	$2.84 \pm .15^{b}$	16	$2.72 \pm .10^{b}$	16	$3.39 \pm .15^{a}$	15	$2.76 \pm .10^{b}$	16	$3.03 \pm .15^{ab}$	16	$2.40 \pm .10^{c}$
		F_1	200	16	$3.80 \pm .18^{a}$			16	$3.19 \pm .18^{b}$			16	$3.05 \pm .18^{b}$		
Kidney wt, g	Kidney	F_0	60	16	$0.53 \pm .02^{a}$	11	$0.75 \pm .04$	16	$0.58 \pm .02^{a}$	13	$0.78 \pm .04^{b}$	16	$0.54 \pm .02^{a}$	13	$0.73 \pm .04^{b}$
		F_1	120	11	$0.53 \pm .02$			15	$0.49 \pm .02$			5	$0.54 \pm .03$		
	Liver	F_0	60	16	$0.58 \pm .02^{a}$	16	$0.76 \pm .03$	16	$0.66 \pm .02^{bc}$	15	$0.80 \pm .03^{c}$	16	$0.56 \pm .02^{a}$	16	$0.71 \pm .03^{c}$
		F_1	200	16	$0.79 \pm .02^{a}$			16	$0.64 \pm .02^{b}$			16	$0.59 \pm .02^{b}$		
Liver/ body wt[5] %	Kidney	F_0	60	16	$7.2 \pm .3^{ac}$	11	$6.2 \pm .5^{bc}$	16	$8.2 \pm .2^{a}$	13	$5.6 \pm .5^{b}$	16	$8.0 \pm .2^{a}$	13	$5.7 \pm .5^{b}$
		F_1	120	11	$7.4 \pm .2$			15	$7.3 \pm .2$				$7.8 \pm .4$		
	Liver	F_0	60	16	$9.3 \pm .3^{a}$	16	$7.2 \pm .2^{b}$	16	$8.3 \pm .3^{a}$	15	$7.3 \pm .2^{b}$	16	$9.0 \pm .3^{a}$	16	$6.9 \pm .2^{b}$
		F_1	200	16	$8.8 \pm .4$			16	$8.4 \pm .4$			16	$7.9 \pm .4$		
Kidney/ body wt %	Kidney	F_0	60	16	$1.6 \pm .1^{a}$	11	$2.2 \pm .1^{b}$	16	$1.7 \pm .1^{a}$	13	$2.0 \pm .1^{b}$	16	$1.7 \pm .1^{a}$	13	$1.9 \pm .1^{b}$
		F_1	120	11	$1.6 \pm .2$			15	$1.5 \pm .2$			5	$1.7 \pm .3$		
	Liver	F_0	60	16	$1.8 \pm .1^{ac}$	16	$2.0 \pm .1^{bc}$	16	$1.7 \pm .1^{a}$	15	$2.1 \pm .1^{b}$	16	$1.6 \pm .1^{a}$	16	$2.0 \pm .1^{bc}$
		F_1	200	16	$1.8 \pm .1^{a}$			16	$1.7 \pm .1^{ab}$			16	$1.5 \pm .1^{b}$		

[a,b,c] Means ± SE on the same line with different superscripts differ significantly ($P < 0.05$)

[1] Centimeters per hectare

[2] Pensacola liquid sludge

[3] Dried Chicago sludge

[4] Bertrand *et al.* (1978)

[5] Calculated by dividing organ weight by final body weight x 100.

TABLE 4. MICE FEEDING TRIAL 1. REPRODUCTIVE DATA OF MICE FED DIETS CONTAINING FREEZE-DRIED KIDNEY AND LIVER TISSUE FROM CATTLE FED A CONTROL DIET, CORN FROM LAND RECEIVING 7.6 CM/HA[1] OF LPS[2] OR 500 G/HEAD/DAY DCS[3,4]

Mice para-meters	Tissue fed	Mice generation	Test days	Control No sludge		7.6 cm/ha LPS		500 g/h/d DCS	
				n	No./litter	n	No./litter	n	No./litter
Number born	Kidney	F_0	60	6	10.1 ± 1.5	7	13.5 ± 1.4	8	13.7 ± 1.3
	Liver	F_0	60	9	10.2 ± 2.1	10	14.9 ± 2.0	7	16.0 ± 2.4
	Liver	F_1	200	16	8.8 ± .7^{a}	16	6.8 ± .7^{b}	11	6.1 ± .7^{b}
Number weaned	Kidney	F_0	60	8	6.1 ± 1.4^{a}	8	8.5 ± 1.4^{a}	8	1.6 ± 1.4^{b}
	Kidney	F_1	120	10	6.4 ± 0.9	15	8.4 ± 0.8	5	6.8 ± 1.4
	Liver	F_0	60	9	5.6 ± 1.5^{a}	10	14.0 ± 1.4^{b}	7	10.9 ± 1.7^{ab}
	Liver	F_1	200	16	8.1 ± .6^{a}	16	6.0 ± .5^{ab}	11	3.9 ± .7^{b}

[a,b] Means ± SE on the same line with different superscripts differ significantly ($P < 0.05$).

[1] Centimeters per hectare.

[2] Pensacola liquid sludge.

[3] Dried Chicago digested sludge.

[4] Bertrand *et al.*

TABLE 5. MICE FEEDING TRIAL 1. MINERAL ANALYSES DATA OF KIDNEY, LIVER, AND MUSCLE TISSUE OF MICE FED FREEZE-DRIED KIDNEY FROM CATTLE FED A CONTROL DIET, CORN FROM LAND RECEIVING 7.6 CM/HA[1] OF LPS[2] OR 500 G/HEAD/DAY DCS[3,4]

Treatment	Mice Generation	Test days	Sex	Cd	Cu	Fe	Co	Zn	Pb	Hg	Cr	Ni	Se
							Mice kidney (mg/kg) wet basis						
Control (no sludge)	F_0	60	F	0.00	4.34	117.1	1.38	18.6	0.77	.069	0.31	2.69	.160
	F_0	60	M	0.00	4.09	121.0	1.42	19.9	0.89	.178	0.53	2.67	.110
	F_1	120	F	-	-	-	-	-	-	-	-	-	-
	F_2	21	F	0.00	2.67	58.8	2.17	18.5	16.7	.000	0.84	5.81	.170
7.6 cm/ha LPS	F_0	60	F	0.00	4.05	105.7	1.13	16.9	0.63	.033	0.19	2.38	.154
	F_0	60	M	0.00	3.62	189.8	1.44	24.4	1.90	.065	0.28	2.84	.140
	F_1	120	F	0.20	3.50	85.0	0.21	16.7	0.07	.070	0.07	0.71	.710
	F_2	21	F	0.00	2.80	49.3	1.72	16.9	1.56	.000	0.23	2.32	.31
500 g/h/d DCS	F_0	60	F	2.35	3.82	109.1	1.21	17.3	0.69	.032	0.37	2.92	.160
	F_0	60	M	1.17	3.96	157.5	1.21	17.9	0.69	.038	0.31	2.71	.120
	F_1	120	F	1.10	3.50	94.0	0.24	19.2	0.12	.120	0.10	1.20	.600
	F_2	21	F	0.00	2.85	49.3	3.93	17.5	3.57	.000	2.50	7.14	.360
							Mice liver (mg/kg) wet basis						
Control (no sludge)	F_0	60	F	0.00	2.55	254.0	0.54	21.1	0.35	.000	0.10	1.14	.003
	F_0	60	M	0.00	4.69	291.0	0.68	21.1	0.56	.014	0.24	1.75	.160
	F_1	120	F	0.10	2.20	154.0	0.08	18.4	0.07	.080	0.10	0.76	.380
	F_2	21	F	0.00	2.37	126.0	0.75	18.3	0.63	.000	0.56	3.75	.19
7.6 cm/ha LPS	F_0	60	F	0.00	3.74	379.1	0.58	41.4	0.36	.004	0.35	1.43	.065
	F_0	60	M	0.00	4.86	247.5	0.55	20.7	0.28	.010	0.05	1.42	.140
	F_1	120	F	0.04	2.60	172.0	0.11	19.8	0.07	.040	0.04	0.35	.360
	F_2	21	F	0.00	2.66	201.8	0.81	19.0	0.58	.000	0.23	2.32	.170
500 g/h/d DCS	F_0	60	F	0.34	2.83	350.0	0.49	20.1	0.25	.006	0.06	1.14	.130
	F_0	60	M	0.23	4.63	268.4	0.56	25.2	0.48	.006	0.10	1.44	.130
	F_1	120	F	0.40	1.80	151.0	0.09	18.1	0.09	.090	0.09	0.89	.450
	F_2	21	F	0.00	2.22	87.6	1.67	20.9	1.39	.000	0.83	2.78	.010

TABLE 5. Continued

Treatment	Generation	Test days	Sex	Cd	Cu	Fe	Co	Zn	Pb	Hg	Cr	Ni	Se
							Mice muscle (mg/kg) wet basis						
Control (no sludge)	F_0	60	F	0.00	1.31	25.2	0.53	13.0	0.37	0.003	.14	1.11	.001
	F_0	60	M	0.00	1.38	27.1	0.47	9.5	0.41	0.006	.16	1.12	.001
	F_1	120	F	0.20	0.19	31.0	0.19	17.7	0.39	.190	.20	1.94	.960
	F_2	21	F	0.00	0.96	17.9	0.96	11.0	1.20	.000	.60	2.49	.006
7.6 cm/ha LPS	F_0	60	F	0.00	1.12	18.1	0.40	10.0	0.34	0.000	.10	1.10	.020
	F_0	60	M	0.00	1.16	28.5	0.46	11.1	0.42	0.000	.11	1.12	.004
	F_1	120	F	0.04	1.10	24.0	0.31	16.5	0.11	.040	.20	0.35	.180
	F_2	21	F	0.00	1.03	18.1	1.04	11.2	1.38	.000	.02	2.76	.000
500 g/h/d DCS	F_0	60	F	0.00	0.92	21.0	0.50	12.6	0.54	0.003	.23	1.88	.007
	F_0	60	M	0.00	1.12	26.4	0.47	10.7	0.42	0.006	.11	1.81	.009
	F_1	120	F	0.90	0.71	28.0	0.62	20.6	0.53	0.090	.40	0.89	.450
	F_2	21	F	0.00	0.58	7.2	0.75	52.2	0.58	.000	.41	1.74	.000

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[1] Centimeters per hectare

[2] Pensacola liquid sludge

[3] Dried Chicago digested sludge

[4] Bertrand *et al.*, 1978

[5] Weanling female mice

TABLE 6. MICE FEEDING TRIAL I. MINERAL ANALYSES DATA OF KIDNEY, LIVER, AND MUSCLE TISSUE OF MICE FED FREEZE-DRIED LIVER FROM CATTLE FED A CONTROL DIET, CORN FROM LAND RECEIVING 7.6 CM/HA[1] LPS[2] OR 500 G /HEAD/DAY DCS[3,4]

Treatment	Mice Generation	Test days	Sex	Cd	Cu	Fe	Co	Zn	Pb	Hg	Se	Cr	Ni
							Mice kidney (mg/kg) wet basis						
Control (no sludge)	F_0	60	F	0.08	2.52	84.0	0.00	16.0	0.00	--	--	0.00	0.00
	F_0	60	M	0.11	3.29	102.0	0.00	19.9	0.00	--	--	0.00	0.00
	F_1	200	F	0.00	3.54	124.8	0.00	17.7	0.00	.024	.300	0.31	0.00
	F_2	21	F	0.06	3.27	92.3	0.00	16.3	0.00	.008	.160	0.14	0.70
7.6 cm/ha LPS	F_0	60	F	0.25	3.28	90.0	0.00	18.4	0.00	--	--	0.00	0.00
	F_0	60	M	0.11	3.25	116.0	0.00	22.4	0.00	--	--	0.00	0.00
	F_1	200	F	0.32	4.00	115.0	0.00	17.5	0.00	.010	.300	0.28	0.50
	F_2	21	F	0.00	2.63	67.2	0.00	9.2	0.00	.008	.210	0.22	0.30
500 g/h/d DCS	F_0	60	F	0.69	2.73	96.0	0.00	17.8	0.00	.60	.300	0.00	0.00
	F_0	60	M	0.32	2.48	80.0	0.00	14.9	0.00	.170	.250	0.00	0.00
	F_1	200	F	1.61	4.00	109.0	1.04	17.6	0.00	--	--	0.00	0.00
	F_2	21	F	0.21	3.60	83.0	1.04	18.7	0.00	--	--	0.00	0.00
							Mice liver (mg/kg) wet basis						
Control (no sludge)	F_0	60	F	0.04	2.94	214.0	0.00	20.6	0.00	--	--	0.00	0.00
	F_0	60	M	0.04	4.55	186.0	0.00	25.5	0.00	--	--	0.00	0.00
	F_1	200	F	0.03	3.23	161.7	0.00	19.5	0.00	.003	.100	0.29	0.00
	F_2	21	F	0.00	19.7	159.8	0.00	16.3	0.00	.008	.020	0.43	0.04
7.6 cm/ha LPS	F_0	60	F	0.04	2.97	161.0	0.00	26.6	0.00	--	--	0.00	0.00
	F_0	60	M	0.04	3.85	224.0	0.00	27.6	0.00	--	--	0.00	0.00
	F_1	200	F	0.03	3.74	204.0	0.00	21.8	0.00	.003	.120	0.14	0.00
	F_2	21	F	0.00	4.90	199.5	0.00	19.9	0.00	.005	.050	0.14	0.00
500 g/h/d DCS	F_0	60	F	0.20	3.05	207.0	0.00	43.2	0.00	.040	.200	0.00	0.00
	F_0	60	M	0.15	3.07	228.0	0.00	23.1	0.00	.040	.400	0.00	0.00
	F_1	200	F	0.43	3.10	230.0	0.36	17.3	0.00	--	--	0.00	0.00
	F_2	21	F	0.29	8.60	201.0	0.36	19.0	0.00	--	--	0.00	0.00

TABLE 6. Continued

Treatment	Mice Generation	Test days	Sex	Cd	Cu	Fe	Co	Zn	Hg	Se	Cr	Ni
							Mice muscle (mg/kg) wet basis					
Control (no sludge)	F_0	60	F	0.00	0.79	22.0	0.00	26.6	--	--	0.00	0.00
	F_0	60	M	0.00	0.81	34.0	0.00	21.4	--	--	0.00	0.00
	F_1	200	F	0.00	1.15	36.3	0.00	20.5	.015	.040	0.40	0.00
	F_2	21	F	0.00	1.33	32.4	0.00	19.1	.008	.030	0.43	0.40
7.6 cm/ha LPS	F_0	60	F	0.00	0.59	21.0	0.00	19.5	--	--	0.00	0.00
	F_0	60	M	0.00	0.72	30.0	0.00	19.7	--	--	0.00	0.00
	F_1	200	F	0.03	1.39	39.6	0.00	22.8	.036	.030	0.36	0.70
	F_2	21	F	0.00	1.36	30.6	0.00	19.7	.010	.020	0.44	0.30
500 g/h/d DCS	F_0	60	F	0.02	0.52	24.0	0.00	26.0	.040	.200	0.00	0.00
	F_0	60	M	0.02	0.68	30.0	0.00	19.4	.040	.200	0.00	0.00
	F_1	200	F	0.04	4.30	316.0	0.35	25.2	--	--	0.35	0.00
	F_2	21	F	0.04	1.40	83.0	0.35	22.2	--	--	0.70	0.00

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[1] Centimeters per hectare

[2] Pensacola liquid sludge

[3] Dried Chicago digested sludge

[4] Bertrand *et al.*, 1978

TABLE 7. MICE FEEDING TRIAL 1. MINERAL ANALYSES BY TREATMENT OF KIDNEY, LIVER, AND MUSCLE TISSUE OF MICE FED FREEZE-DRIED KIDNEY OR LIVER TISSUE FROM CATTLE FED A CONTROL DIET, CORN FROM LAND RECEIVING 7.6 CM/HA[1] LPS[2] OR 500 G/HEAD/DAY DCS[3] [4]

Treatment	Mice Generation	Test days	n	Cd	Cu	Fe	Co	Zn	Pb
						Mice kidney (mg/kg) wet basis[5]			
Control, 0%	F_0	60	4	0.04±.20[6]	3.56±.13	106.0±8.9	0.70±.03	18.60±.37	0.415±.140
7.6 cm/ha LPS	F_0	60	4	0.09±.20	3.55±.13	125.3±8.9	0.64±.03	20.52±.87	0.632±.140
500 g/h/d DCS	F_0	60	4	1.13±.20[a]	3.24±.13	110.6±8.9	0.60±.03	16.97±.37	0.345±.140
						Mice liver (mg/kg) wet basis[5]			
Control, 0%	F_0	60	4	.020±.021	3.68±.21	236.2±19.0	0.30±.01	22.07±3.35	0.227±.034
7.6 cm/ha LPS	F_0	60	4	.020±.021	3.85±.21	252.9±19.0	0.28±.01	29.07±3.35	0.160±.034
500 g/h/d DCS	F_0	60	4	.230±.021[b]	3.39±.21	263.3±19.0	0.26±.01	27.90±3.35	0.182±.034
						Mice muscle (mg/kg) wet basis[5]			
Control, 0%	F_0	60	4	.000±.002	1.07±.02[c]	27.07±.92	0.25±.84	17.62± .84	0.195±.020
7.6 cm/ha LPS	F_0	60	4	.000±.002	0.89±.02	24.40±.92	0.21±.84	15.07± .84	0.190±.020
500 g/h/d DCS	F_0	60	4	.010±.002	0.81±.02	25.35±.92	0.24±.84	17.17± .84	0.240±.020

[1] Centimeters per hectare

[2] Pensacola liquid sludge

[3] Dried Chicago digested sludge

[4] Bertrand *et al.*, 1978

[5] Entries are diet means averaged across sex and feed types which were 5% kidney and 10% liver

[6] Means ± SE

[ab] Cd was higher at the 500 g/head/day DCS treatment ($P < 0.05$)

[c] Cu in mice muscle was higher in the controls ($P < .05$).

TABLE 8. MICE FEEDING TRIAL 1. MINERAL ANALYSES BY TREATMENT OF KIDNEY, LIVER, AND MUSCLE TISSUE OF MICE FED FREEZE-DRIED KIDNEY FROM CATTLE FED A CONTROL DIET, CORN FROM LAND RECEIVING 7.6 CM/HA[1] LPS[2] OR 500 G/HEAD/DAY DCS[3] [4]

Treatment	Mice Generation	Test days	n	Hg	Se	Cr	Ni
					Mice kidney (mg/kg) wet basis[5]		
Control	F_0	60	2	.123±.054[6]	.135±.024	.42±.10	2.68±.01
7.6 cm/ha LPS	F_0	60	2	.049±.015	.147±.006	.23±.04	2.61±.22
500 g/h/d DCS	F_0	60	2	.035±.002	.140±.019	.34±.02	2.81±.10
					Mice liver (mg/kg) wet basis[5]		
Control	F_0	60	2	.007±.006	.081±.078	.17±.06	1.44±.30
7.6 cm/ha LPS	F_0	60	2	.007±.002	.102±.037	.20±.14	1.42±.00
					Mice muscle (mg/kg) wet basis[5]		
500 g/h/d DCS	F_0	60	2	.006±.000	.130±.000	.08±.01	1.29±.14
Control	F_0	60	2	.004±.001	.004±.000	.15±.01	1.11±.00
7.6 cm/ha LPS	F_0	60	2	.000±.000	.014±.008	.10±.00	1.11±.01
500 g/h/d DCS	F_0	60	2	.004±.001	.008±.001	.17±.05	1.44±.36

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[1] Centimeters per hectare

[2] Pensacola liquid sludge

[3] Dried Chicago digested sludge

[4] Bertrand *et al.*, 1978

[5] Entries are diet means averaged across sex

[6] Mean ± SE

TABLE 9. MICE FEEDING TRIAL 1. MINERAL ANALYSES BY SEX OF KIDNEY, LIVER, AND MUSCLE TISSUE OF MICE FED FREEZE-DRIED KIDNEY OR LIVER TISSUE FROM CATTLE FED A CONTROL DIET, CORN FROM LAND RECEIVING 7.6 CM/HA[1] LPS[2] OR 500 G/HEAD/DAY DCS[3] [4]

Sex	Mice Generation	Test days	n	Cd	Cu	Fe	Co	Zn	Pb
						Mice kidney (mg/kg) wet basis[5]			
Female	F_o	60	6	0.56±.20[6]	3.45±.12	100.3±8.9	0.62±.03	17.50 ± .87	0.348±.140
Male	F_o	60	6	0.28±.20	3.44±.13	127.7±8.9	0.67±.03	19.90 ± .87	0.580±.140
						Mice liver (mg/kg) wet basis[5]			
Female	F_o	60	6	0.10±.02	3.01±.21	260.8±19.0	0.26±.01	28.83 ± 3.35	0.160±.014
Male	F_o	60	6	0.07±.02	4.27±.21[a]	240.8±19.0	0.29±.01	23.86 ± 3.35	0.220±.014
						Mice muscle (mg/kg) wet basis[5]			
Female	F_o	60	6	0.003±.002	0.87±.02	21.38± .92	0.23±.01	17.95 ± .84	0.209± .02
Male	F_o	60	6	0.003±.002	0.97±.02[b]	29.33± .92[b]	0.23±.01	15.30 ± .84	0.208± .02

[1] Centimeters per hectare

[2] Pensacola liquid sludge

[3] Dried Chicago digested sludge

[4] Bertrand *et al.*, 1978

[5] Entries are diets means averaged across feed types and treatment groups

[6] Means ± SE

[a] Cu in male mice liver was greater (P < 0.01) than in females

[b] Cu and Fe in male mice muscle was greater (P < 0.01) than in females

MICROBIOLOGY

E. M. Hoffmann and Suzanne Hickman

FEEDING STUDIES ON ANIMALS: GENERAL PROCEDURES

Blood and feces were examined monthly from animals fed on sludge-amended diets or on feed material which was grown on land fertilized with sewage sludge. At the termination of the feeding experiments, the animals were slaughtered, and kidney, liver, and spleen samples were also examined. Only tissue samples were analyzed in experiments involving poultry. All bacteriologic procedures were carried out in accordance with the Manual of Clinical Microbiology, second edition, American Society of Microbiology (1) and Baily and Scott's Diagnostic Microbiology (2).

BLOOD CULTURES

Two ml of blood were drawn from the jugular veins of cattle or from the carotid arteries of swine into 18 ml of peptone broth using vacutainer blood culture tubes (Becton-Dickinson, Rutherford, NJ). The culture tubes were vented, and placed at 37°C. Observations for growth were made daily. Gram stains were made from tubes showing turbidity, and they were also subcultured onto blood agar plates. The plates were incubated at 37°C, and observed for growth. Primary blood culture tubes were kept for 2 weeks before discarding.

All bacterial colonies which appeared on blood plates were gram stained. Gram positive cocci which grew in clumps were subjected to additional tests (listed below) to determine if they were *Staphylococcus aureus*. Likewise, gram positive cocci growing in chains (or pairs) and gram negative rods were tested to determine if they were potentially pathogenic *Streptococci* or enteric pathogens, respectively.

Method of Analysis

Gram positive cocci in clumps

1. Coagulase tests
2. Catalase tests
3. Mannitol fermentation
4. Tellurite reduction on Vogel-Johnson medium
5. Hemolysis on 5% blood agar

Gram positive cocci in chains

1. Hemolysis on 5% blood agar (aerobic and anaerobic)
2. Sensitivity to bacitracin (0.04 units)
3. Sensitivity to optichin
4. Serologic grouping: β-hemolytic bacteria sensitive to 0.04 units of bacitracin were grouped using specific antiserum to the group A antigen

Gram negative rods

1. Fermentation of glucose, sucrose, and lactose using triple sugar iron agar (TSI agar)
2. Urease production
3. Pyruvate production from phenylalanine
4. H_2S production
5. Bacteria which failed to ferment sucrose or lactose but which fermented glucose, did not produce urease, and did not convert phenylalanine to pyruvic acid were subjected to additional testing using Roche "Enterotubes" (Enterotube II, Roche Diagnostics, Nutley, NJ). The following tests are made with this system:
   a. Acid and gas from dextrose
   b. Decarboxylation of lysine
   c. Decarboxylation of ornithine
   d. H_2S production
   e. Production of indole from tryptophane
   f. Adonitol fermentation
   g. Lactose fermentation
   h. Arabinose fermentation
   i. Sorbitol fermentation
   j. Acetoin production
   k. Deamination of phenylalanine
   l. Urease production
   m. Citrate utilization
6. Final identification of *Salmonella* and *Shigella* was accomplished using specific antisera (Lee Laboratories, Grayson, GA)

FECAL SAMPLES

Fecal samples were streaked directly on XLD and SS agar plates. Approximately 1.0 g amounts of feces were also placed into tetrathionate broth. The media were incubated for 20-24 hours at 37°C. All of the tetrathionate cultures were subcultured onto XLD and SS agar plates, and the plates were incubated for another 20-24 hour period at 37°C. On XLD medium, *Salmonella* bacteria usually produce red colonies with black centers, however, some *Salmonella* give red colonies without black colonies. *Shigella* bacteria also give red colonies on the medium. Lactose fermenting bacteria give red colonies on SS medium whereas non-lactose fermenting enteric bacteria (potentially pathogenic) have non-pigmented colonies. All suspicious colonies were picked and subjected to preliminary screening according to the procedure described for treatment of gram negative rods isolated from blood cultures. Bacteria which failed to ferment sucrose or lactose but which fermented glucose, were urease negative, and did not deaminate phenylalanine, were placed on Roche "Enterotubes" for identification. Final identification of *Salmonella* or *Shigella* was accomplished using specific antisera. *Salmonella* were placed into serologic groups, but it was possible to identify the species of *Shigella*.

ORGAN SAMPLES

Organ samples were obtained from animals during slaughter. Samples of spleen, liver, and kidney were obtained aseptically at the slaughterhouse immediately after the animals were opened. A separate sterilized knife was used for each sample, and the organs were handled only with sterile gloves. The samples were placed in sterile jars and transported to the laboratory for culturing.

The tissues were minced with sterile scissors and approximately 5 g amounts were placed in fluid thioglycholate broth (with glucose and indicator). A small amount of minced tissue was also placed on Lowenstein-Jensen medium. The media were incubated at 37°C. Fluid thioglycholate cultures were observed after 24 and 48 hours. Lowenstein-Jensen cultures were kept for 4 weeks, and examined daily for the appearance of suspicious colonies. Gram stains were performed on bacteria appearing in the fluid thioglycholate tubes, and additional tests were performed on selected types of bacteria. Gram positive cocci growing in clumps, gram positive cocci growing in chains (or pairs), and gram negative rods were subjected to additional testing according to the protocol described for bacteria isolated from blood cultures.

BACTERIOLOGICAL ANALYSIS OF FEED, GRASS, AND SLUDGE

The feed, dried sludge used for diet amendment, wet sludge used for soil treatment, and grasses grown on sludge-fertilized soil were subjected to analysis for pathogenic enteric bacteria. In addition, the numbers of coliform and fecal coliform bacteria were estimated.

One gram quantities of each sample were placed in tetrathionate broth and incubated at 37°C. The cultures were then subcultured onto XLD and SS agar plates which were incubated at 37°C for 20-24 hours. Suspicious colonies (see preceding section on Fecal Samples) were picked and subjected to further analysis using the procedures that have been listed in the section dealing with the treatment of gram negative rods isolated from blood cultures.

Five gram quantities of the test materials were placed into 100 ml volumes of sterile water. Grass samples were cut into fine pieces using sterile scissors before being added to the water. The mixtures were shaken according to the procedure described in Standard Methods for the Examination of Water and Wastewater (3) and the numbers of coliform bacteria per 100 ml volume were estimated using the most probable number method (MPN) of analysis (3). Replicate samples (totaling 5) of 10 ml, 1.0 ml, and 0.1 ml were taken from the 100 ml volumes of water in which the various samples had been suspended. Fecal coliforms were also enumerated using the MPN procedure with EC incubated at 44.5^{o}C (3).

BACTERIOLOGICAL ANALYSIS OF GROUND WATERS, SURFACE WATERS, AND THE SLUDGE LAGOON

Sludge from the holding pond (lagoon) at the Jay Agricultural Research Center, water from ponds and streams draining sludge-treated fields, and water from wells drilled in the test area were analyzed for numbers of coliform and fecal coliform bacteria. Samples were submitted monthly. Those samples which were turbid, or which had particulate matter were analyzed using the MPN methods for total coliforms and fecal coliforms (3). Clear water samples were subjected to the membrane filter technique for estimation of total coliforms and fecal coliforms (3).

SOIL ADSORPTION STUDIES: GENERAL PROCEDURES

Limited studies were conducted to study the fate of *Escherichia coli* in the soil-water system at the Jay Agricultural Research Center. For these studies, a double antibiotic resistant mutant of *E. coli* (streptomycin and ampicillin resistant) was selected from the sewage sludge used for soil amendment on the farm at the Jay center. Five types of experiments were conducted.

Extent of *E. coli* adsorption to soil
Rate of *E. coli* adsorption to soil
Movement of *E. coli* through topsoil
Elution of *E. coli* from topsoil
Survival of *E. coli* in the equivalent of ground water

ANTIBIOTIC RESISTANT *E. COLI* MUTANT

A 1.0 g samples of Pensacola sewage sludge was cultured in tetrathionate broth for ca - 24 hours at 37^{o}C. This culture was then subcultured onto eosin methylene blue agar (EMB), and incubated again for ca - 24 hours at 37^{o}C. A lactose fermenting colony which demonstrated a greenish metallic sheen was selected and cultured on an Enterotube II at 37^{o}C for ca - 24 hours. The isolate, identified as *Escherichia coli*, was transmitted to Dr. D. E. Duggan (Department of Microbiology and Cell Science, University of Florida), who selected a mutant which was resistant to both streptomycin (200 μg/ml), and ampicillin (20 μg/ml). All of the experiments described in the section dealing with soil adsorption utilized this antibiotic resistant mutant of *Escherichia coli*, and it is referred to simply as *E. coli*.

EXTENT OF *E. COLI* ADSORPTION TO SOIL

Studies were carried out to determine the numbers of *E. coli* that would adsorb to a given amount of Orangeburg topsoil (from the Jay ARC) in a set period of time. For some experiment, the soil and bacteria were suspended in 0.01 M $CaCl_2$ to approximate the ionic conditions that would be encountered in a soil-ground water system. Other experiments were conducted with deionized water to approximate the conditions of soil suspended in rain water.

Soil from the top 15.0 cm was collected from a field at the Jay Agricultural Center which had been recently cleared of trees. This area had not been planted to crops or fertilized in the past 20 years or longer. The soil was air dried (20-24°C) and sifted through a screen such that the particles were equal to or less than 1.0 mm in diameter.

The earlier adsorption studies were carried out by suspending various amounts of soil in 100 ml of a standardized suspension of *E. coli* in water or 0.01 M $CaCl_2$. The soil-bacteria mixtures were shaken in Erlynmeyer flasks at about 60 passages per minute at room temperature (22-24°C). After 60 minutes, the soil was sedimented by centrifugation at about 125 g at room temperature. The supernatant fluids were collected, and the number of *E. coli* enumerated by plating in nutrient agar containing 200 µg/ml streptomycin and 20 µg/ml ampicillin. A control consisted of bacteria in water or 0.01 M $CaCl_2$ held for 60 minutes at room temperature without the addition of soil.

Later, static adsorption experiments were carried out using 1 part of soil to 2 parts of sterile $CaCl_2$. The mixtures were placed in screw-capped tubes and mixed slowly by repeated inversion for 2 hours at room temperature on a Fisher Roto-Rack 343 shaker. The rate of mixing was about 20 inversions per hour. As indicated above, the initial studies of this type utilized bacteria suspended in water. Experiments were also conducted with *E. coli* suspended in 0.01 M $CaCl_2$ and in various concentrations of Pensacola sewage sludge to determine the effects of the suspending medium or the extent of bacterial adsorption to soil.

We also examined the elution of trapped and adsorbed *E. coli* from Orangeburg soil under the equivalent of ground water conditions. Time did not permit varying the physical conditions of the system to test the effect on desorption and/or elution. *Escherichia coli* were adsorbed to the soil using 0.01 M $CaCl_2$ as the suspending medium with 2 parts of bacterial suspension to 1 part soil. Adsorption was for 2 hours at 22-24°C with mixing by slow inversion (as described previously). The mixture was rapidly filtered using a Gelman 47 mm magnetic filter holder, and a disk of Whatman No. 1 filter paper. A control consisted of bacteria in 0.01 M $Ca Cl_2$ without soil. These were also filtered, and the base-line input level was taken as the number of bacteria recoverable from this control at time 0.

The soil which was trapped by the filter paper disk was then washed with 0.01 M CaCl . Plate counts were performed on 25 ml volumes of filtrate using the membrane filtration technique and nutrient agar plus antibiotics (200 g/ml streptomycin and 20 g/ml ampicillin). The control filter paper disk was also washed in the same fashion, and plate counts were performed.

The number of *E. coli* before mixing with soil were determined by plate counting on nutrient agar plus antibiotics. The numbers of bacteria bound (or trapped) during the adsorption steps were calculated by taking the difference between the initial count and the count in the first filtrate after a correction had been made to account for bacteria nonspecifically adsorbed or trapped in the filtration system. The elution of *E. coli* from the soil was then estimated by summarizing the counts made of the water wash fractions.

BACTERIOLOGY: CONCLUSIONS

Blood samples were obtained from cattle which were fed on a diet which was amended with dried sewage sludge. Blood was taken monthly, and bacteria were isolated from a high percentage of these samples even though clinical evidence indicated that there was no disease. These bacteria were not readily identified as pathogens, and they probably represented contamination introduced at the time of sampling. The sampling conditions were not conducive to obtaining uncontaminated materials. One isolation of *Staphylococcus aureus* was made from a single animal in the sludge trial group (Table 1). Similar results were obtained from blood and tissue samples from swine (data not shown).

Fecal specimens from the sludge fed cattle were tested for the presence of enteric pathogens. A total of 5 *Salmonella* isolations were made. All of these were from the sludge fed group which received 500 g per head per day. Three of the isolates were *Salmonella enteritidis* group A, and 2 were *Salmonella enteritidis* group B (Table 2). Similar results were obtained from fecal samples obtained from swine (data not shown).

Kidney, liver, and spleen samples were taken at the time of slaughter, and examined for the presence of bacteria. Bacteria were isolated from a high percentage of the samples, but very few of these were identified as pathogens when examined by the criteria listed in the procedures section. The microorganisms were probably contaminants introduced into the tissues at the time of slaughter since the conditions were not ideal for bacteriologic sampling.

One isolation of *Staphylococcus aureus* was obtained from an animal in the control group which did not receive sludge in the diet. Three cases were found where β-hemolytic streptococci were present. These bacteria were not *Staphylococcus pyogenes* (Table 3).

Bacteriologic analyses were conducted with 1 poultry trial. It was very difficlut to obtain "clean" poultry samples, and we abandoned attempts to isolate any bacteria other than enteric pathogens. *Salmonella enteritidis* group C was recovered from 2 fecal samples. One of these samples was from a male chicken fed on 50% sludge-grown grain, and the other was from a female chicken fed on 100% sludge-grown grain (Table 4).

Feed, forage, and dried sludge used for supplementation of diets were analyzed for enteric pathogens, fecal coliforms, and total coliforms. There were no enteric pathogens isolated using our methods. Fecal coliform counts were negligible except in feed, and sludge amended feed from the Live Oak ARC in 1978 (Table 5). Two samples from the Jay ARC had substantial total coliform counts in 1976, and there were elevated total coliform counts associated with the materials obtained from the Live Oak ARC in 1977 (Table 5).

Fecal and non-fecal coliform analyses were carried out using water samples and from the Jay ARC. These samples were from wells and bodies of water in the vicinity of the sludge storage area, and the fields which were amended with sludge. The data summarizing these analyses for 1976 and 1977 are found in Table 6. Data from 1978 are located in Table 7. Substantial numbers of non-fecal coliforms began appearing in August and continued through October. Fecal coliforms began appearing in some samples in September, and in some cases they persisted into November. Results of fecal and non-fecal coliform analysis of water samples for 1979 are found in Table 8. Both fecal and non-fecal coliforms were associated with certain samples throughout the period of testing.

Fecal and total coliform counts were made on a variety of sludges. The results of the tests are shown in Table 9. The material from the sludge pond was used to amend the soil of the Jay ARC for certain trials which were conducted as part of the project. It can be seen that the fecal coliform numbers dropped to a very low level during the period between May, 1978 and November, 1978. The possible significance of this will be addressed in a paper which is being prepared in collaboration with Dr. S. Farrah and Dr. G. Bitton. Both of these researchers are co-investigators on this project.

SOIL STUDIES: BACTERIOLOGY

A mutant strain of *Escherichia coli* (resistant to streptomycin and ampicillin) was obtained from Dr. Dennis Duggan. This organism was maintained in streptomycin (200 μg/ml), and ampicillin (20 μg/ml) in nutrient broth. The growth characteristics of this strain were determined at 35°C in the above medium. Most of the experiments in this study employed mid-log phase bacteria which usually had an optical density of 0.600-0.650 at a wave length of 550 nm.

Adsorption experiments were conducted using *E. coli* suspended in water with different concentrations of soil. It was found that roughly 90% of the bacteria were removed from the fluid phase by a 10% soil suspension. The mixture was shaken at 23°C for 60 minutes before analysis.

The rate of adsorption of *E. coli* to soil was measured using a 10% soil suspension at about 23°C. The suspending medium was water. Maximum adsorption occurred at about 40 minutes under these conditions. It was interesting to see that there was marked bacterial replication in the control which consisted of *E. coli* suspended in water. Again, there was better than 90% maximum removal of the bacteria from the fluid phase when compared to the control.

There was less adsorption of the mutant strain of *E. coli* to soil when the bacteria and soil were suspended in 0.01 M $CaCl_2$. Roughly 64% of the input level was removed from the fluid phase by a 10% soil suspension. However, more adsorption was achieved in 0.01 M $CaCl_2$ when the ratio of bacterial suspension to soil was changed to 2:1. Under these conditions, about 90% removal occurred in 60 minutes at 22-24°C. In this experiment, the bacteria in the control apparently did not replicate during the time-course of the experiment. This was seen in each of 3 experiments. The adsorption rate experiments carried out with water always showed an increase in bacterial number during the time-course of the experiment. Three experiments were carried out. We have no ready explanation for these different results.

Bacterial loading capacity of the Orangeburg type soil in water was investigated. The objective of this experiment was to determine the maximum number of bacteria that could be bound (per gram) to this type soil. It was found that nearly all of the bacteria added, at any concentration, were removed from the fluid phase from the soil, and the expected plateau was not reached. A number of technical problems prevented us from determining the total loading capacity of the Orangeburg soil for *E. coli*. It was interesting to note the loading capacity of the Orangeburg soil for *E. coli*. There was a linear relationship between the amount of bacteria added to the system and the number adsorbed.

Organic matter, in the form of sewage sludge, did not seem to influence the extent of adsorption of *E. coli* to Orangeburg soil. Sludge concentrations up to 4% did not have an appreciable effect on the ability of the soil to adsorb bacteria.

The strong adsorption of *E. coli* to soil was further demonstrated by failure of the bacteria to elute from the particles when washed with the suspending medium. Only 0.01 M $CaCl_2$ was used in these experiments. Very few bacteria could be recovered from a 2 g sample of soil which had adsorbed about 5.2×10^6 bacteria. This suggests that the bacteria were indeed bound to the soil and not trapped in pores and between particles. Other experiments were carried out to determine the conditions necessary for eluting the bacteria from the soil. No evidence for elution could be found by increasing ionic strength or by alteration of pH (data not shown).

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TABLE 1. SLUDGE FEEDING TRIALS, CATTLE, JAY ARC BLOOD CULTURES

Dates	Treatment	Total Samples	Samples with bact. growth	Pathogenic isolates*
121975	Control	28	19	0
to	Liquid digested sludge - 1	28	19	1, *S. aureus*
051576	Liquid digested sludge - 2	28	19	0
081976	Control	24	11	0
to	Sludge diet (250 g/h/d)	24	8	0
121676	Sludge diet (500 g/h/d)	24	10	0
032377	Control corn	56	52	0
to	Control corn + sludge	56	53	0
081177	Sludge corn	56	52	0
022278	Control	40	19	0
to	Liquid digested sludge - 1	40	22	0
080978	Liquid digested sludge - 2	40	16	0
052379	Control	56	14	0
to	Liquid digested sludge - 1	56	26	0
110779	Liquid digested sludge - 2	56	31	0

* Pathogens defined according to the criteria described in the procedures section.

TABLE 2. SLUDGE FEEDING TRIALS, CATTLE, JAY ARC FECAL SAMPLES

Dates	Treatment	Total Samples	Pathogens Isolated[a]
121975			
to			
051576		No samples	
081976	Control	40	0
to	Sludge diet (250 g/h/d)	40	0
121676	Sludge diet (500 g/h/d)	40	3[b]
032377	Control corn	56	0
to	Control corn + sludge	56	0
081177	Sludge corn	56	0
022278	Control	56	0
to	Liquid digested sludge - 1	56	0
080978	Liquid digested sludge - 2	56	2[c]
052379	Control	56	0
to	Liquid digested sludge - 1	56	0
110779	Liquid digested sludge - 2	56	0

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[a] Enteric pathogens identified according to methods described in the procedures section.

[b] *Salmonella enteritidis*, group C. Isolated from different individuals.

[c] Two isolates from different individuals. *Salmonella enteritidis*, group A, and *Salmonella enteritidis*, group B.

TABLE 3. SLUDGE FEEDING TRIALS, CATTLE, JAY ARC NECROPSY CULTURES[a]

Dates	Treatment	Kidneys[b]	Liver[b]	Spleens[b]
121975	Control	1/8	0/8	1[c]/8
to	Liquid digested sludge - 1	0/8	1/8	0/8
101576	Liquid digested sludge - 2	2/8	4/8	3/8
081976	Control	4/8	1/8	6/8
to	Sludge diet (250 g/h/d)	3/8	2/8	2/8
121676	Sludge diet (500 g/h/d)	2/8	1/8	0/8
032377	Control corn	4/8	6[d]/8	3[e]/8
to	Control corn + sludge	6/8	4/8	3/8
081177	Sludge corn	5/8	4/8	0/8
052379	Control	5/8	4/8	3/8
to	Liquid digested sludge - 1	4/8	3/8	5/8
110779	Liquid digested sludge - 2	5/8	5/8	4/8

---

[a] Organ samples cultured in fluid thioglycholate broth, tetrathionate broth, and Lowenstein-Jensen medium. Pathogens, when present, were identified according to the protocols described in the procedures section.

[b] The denominator is the total number of samples. The numerator is the number of samples with bacteria present.

[c] *Staphylococcus aureus*

[d] Two isolations of gram positive, β-hemolytic streptococci which were non-groupable with existing antisera, and which were bacitracin-resistant.

[e] One isolate same as above.

TABLE 4. POULTRY FEEDING TRIAL, GAINESVILLE FECAL AND BLOOD SAMPLES[a]

Date	Treatment	Feces[b]	Blood[b]
102876	Control M	0/5	0/5
102876	Control F	0/5	2[c]/5
102876	50% M	1[d]/5	0/5
102876	50% F	0/5	0/5
102876	100% M	0/5	0/5
102876	100% F	1[d]/5	0/5

[a] Samples analyzed for enteric pathogens only.

[b] The denominator is the total number of samples, the numerator is the number of samples with enteric bacteria present.

[c] *Proteus vulgaris*

[d] *Salmonella enteritidis*, group C, Vi negative

TABLE 5. BACTERIAL ANALYSIS - FEED, FORAGE, AND DRIED SLUDGE SUPPLEMENTS

Location	Code	Year	Enteric Pathogens[a]	Coliforms Fecal	Coliforms Total[b]
Jay ARC	Conc. supplement	1976	0/2	-	< 2/2
Jay ARC	Ground corn	1976	0/2	-	2700/2
Jay ARC	Corn shucks	1976	0/2	-	1200/2
Jay ARC	Control	1976	0/1	-	< 2/1
Jay ARC	10% sludge	1976	0/1	-	< 2/1
Jay ARC	20% sludge	1976	0/1	-	< 2/1
Gainesville	Control feed	1977	0/7	3.3/7	29/7
Gainesville	50% Chicago sludge	1977	0/7	< 2/7	1.4/7
Gainesville	Dried Chicago sludge	1977	0/7	< 2/7	1.4/7
LiveOak ARC	ASP control	1977	0/3	.7/3	6/3
LiveOak ARC	ASP 10% sludge	1977	0/3	3/3	4.3/3
LiveOak ARC	ASP20% sludge	1977	0/3	.7/3	8/3
LiveOak ARC	Lincomycin control	1977	0/4	< 2/4	22/4
LiveOak ARC	Lincomycin 10% sludge	1977	0/4	.5/4	6.8/4
LiveOak ARC	Lincomycin 20% sludge	1977	0/4	3.5/4	.5/4
LiveOak ARC	Dried sludge	1977	0/4	9/4	66/4
LiveOak ARC	Control	1978	0/8	119/8	143/8
LiveOak ARC	10% sludge	1978	0/8	74/8	644/8
LiveOak ARC	20% sludge	1978	0/8	4/8	233/8
Jay ARC	Control silage	1978	0/5	< 2/5	< 2/5
Jay ARC	Conc. supplement	1978	0/5	118/5	83/5
Jay ARC	6 Ac. In. silage	1978	0/5	< 2/5	< 2/5
Jay ARC	9 Ac. In. silage	1978	0/5	< 2/5	< 2/5
Ona ARC	Control feed	1973	0/3	4.6/3	4.6/3
Ona ARC	Manure sludge	1978	0/3	180/3	180/3
LiveOak ARC	F2 control	1978	0/4	732/4	2200/4
LiveOak ARC	F2 10% sludge	1978	0/4	1224/4	2030/4
LiveOak ARC	F2 20% sludge	1978	0/4	1263/4	1602/4
LiveOak ARC	F3 control	1978	0/2	1/2	13/2
LiveOak ARC	F3 10% sludge	1978	0/2	800/2	1470/2
LiveOak ARC	F3 20% sludge	1978	0/2	466/2	>2400/2
LiveOak ARC	Pigs control	1978	0/1	34/1	>2400/1
LiveOak ARC	Pig sludge corn	1978	0/1	< 2/1	>2400/1
Ona ARC	Silage	1979	0/18		23/18

TABLE 5. Continued

Location	Code	Year	Enteric Pathogens[a]	Coliforms Fecal	Coliforms Total[b]
LiveOak ARC	F2 Control	1979	0/1	130/1	>2400/1
LiveOak ARC	F2 10% sludge	1979	0/1	>2400/1	>2400/1
LiveOak ARC	F2 20% sludge	1979	0/1	>2400/1	>2400/1
LiveOak ARC	$F_2$1 control	1979	0/1	2/1	2/1
LiveOak ARC	$F_2$1 sludge corn	1979	0/1	< 2/1	34/1
Jay ARC	Grass 54 E	1979	0/4	1/4	611/4
Jay ARC	Grass 54 W	1979	0/4	.5/4	602/4
Jay ARC	Grass 53 W	1979	0/4	.5/4	603/4
Jay ARC	Grass 53 E	1979	0/4	2.3/4	605/4
Jay ARC	Grass 59 E	1979	0/4	.5/4	612/4
Jay ARC	Grass 59 W	1979	0/4	.5/4	603/4
Jay ARC	Sludge (pond)	1979	0/4	326/4	13000/4

[a] Denominator is the total number of samples. The numerator is the number containing enteric pathogens.

[b] Denominator is the total number of samples. The numerator is the average coliform count per gram for the group.

TABLE 6. FECAL AND NON-FECAL COLIFORM COUNTS FROM WATER SAMPLES OBTAINED MONTHLY FROM THE JAY ARC[a], 1976-1977

	1976			1977			
	10-5[b]	11-2[c]	12-7	1-4	2-2	3-2	4-5
Northeast well	0	0/0	0/0	0/0	0/0	0/0	0/0
Middle well	0	0/0	0/0	0/0	0/0	0/0	0/0
Southwest well	0	0/0	0/0	0/0	0/0	0/0	0/0
Sludge pond well	0	0/0	0/0	0/0	0/0	0/0	0/0
Spring	3	0/0	2/3	0/0	0/0	0/1	5/4
Pond creek	0	4/0	2/150	1/0	17/3	25/15	0/ 300
Well w/o pump	2	0/0	0/0	17/2	0/0	0/0	0/0
A-1	0	0/0	160/140	0/0	0[b]	0/0	0/0
A-2	0	0/0	0/0	0/0	0[b]	32/260	0/0
B-1	0	0/100	0/0	0/0	2[b]	0/0	0/0
B-2	0	0/0	0/80	0/0	0[b]		0/0

	1977							
	5-3	6-7	7-6	8-3	9-7	10-5	11-2	12-7
Northeast well	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Middle well	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Southwest well	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Sludge pond well	0/0	0/0	0/0	0/0	0/0	0/0	0/0	-
Spring	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Pond creek	1/3	3/6	109/30	2/50	0/0	7/0	0/0	11/5
Well w/o pump	0/0	5/7	1/4	0/0	0/0	12/16	0/58	0/0
A-1	0/0	0/0	0/0	-	-	-	-	-
A-2	0/0	0/0	0/0	-	-	-	-	-
B-1	0/0	-	0/0	-	-	-	-	-
B-2	0/0	-	0/0	-	-	-	-	-

[a] Membrane filtration procedure used for analysis. [b] Non-fecal counts only. Samples were 100 ml

[c] Non-fecal and fecal counts on 100 ml samples. Numerator=non-fecal counts; denominator=fecal counts.

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TABLE 7. FECAL AND NON-FECAL COLIFORM COUNTS FROM WATER SAMPLES OBTAINED FROM THE JAY ARC[a], 1978

	1-4	2-8	4-3	5-3	6-5	7-10	8-9	9-11	10-4	11-1	12-6
Northeast well	0/0	0/0	LA[c]	0/0	0/0	-	TNTC/0	TNTC/13	TNTC/0	-	0/0
Middle well	0/0	TNTC	LA[c]	0/0	0/0	LA	TNTC/0	TNTC/0	<2[b]/<2	0/0	0/0
Southwest well	0/0	TNTC	LA[c]	-	0/0	LA	-	TNTC/1	0/0	1/0	0/0
Sludge pond well	0/0	-	LA[c]	29/1	0/0	86/2	TNTC/0	5/1	2/0	3/0	0/0
Hill top well	0/0	0/0	LA[c]	0/0	0/0	LA	TNTC/0	170[b]/<2	8/0	1/4	-
Spring	0/1	-	LA[c]	3/1	2/16	LA	TNTC	TNTC	220[b]/49	70[b]/<2	0/1
Pond creek	0/51	11/23	LA[c]	8/3	0/3	42/1	-	TNTC	50/100	79[b]/13	<2[b]/<2
Well w/o pump	0/0	0/0	LA[c]	3/1	0/0	LA	TNTC	-	5[b]/<2	44[b]/<2	-
3-FC[b]	0/0	8/0	LA[c]	7/19	5/4	33/13	< 2	33/33	8/5	2400[b]/ 2	70[b]/920
6-FC[b]	0/0	0/0	LA[c]	3/0	3/2	11/7	< 2	170/130	2/<2	-	<2[b]/8

[a] Membrane filtration procedure used for analysis. Non-fecal and fecal counts. Numerator = non-fecal counts; denominator = fecal counts.

[b] MPN procedure used when samples were turbid.

[c] LA = lab accident. Samples leaked out during transport.

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TABLE 8. FECAL AND NON-FECAL COLIFORM COUNTS FROM WATER SAMPLES OBTAINED FROM THE JAY ARC[a], 1979

Sample Source	1-10	2-12	3-7	5-2	7-10	8-7
Northeast well	0	0	0	0	0	0
Middle well	0	0	0	0	0	0
Southeast well	0	0	0	1	0	2
Sludge pond well	0	0	0	0	0	0
Hill top well	0	0	0	0[b]/0	0	0
Spring	22	48	8	1	28	24
Pond creek[b]	180/170	46/46	3/5	2	-	122
Well w/o pump[b]	<2/<2	75/4	49/<2	8/<2	337/13	>2400/8
3-FC[b]	-	9/<2	<2/<2	44/5	-	218/23
6-FC[b]	-	>2400/23	5/<2	5/<2	535/5	>2400/23

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[a] Membrane filtration procedure used for analysis. Fecal counts only.

[b] MPN procedure used when samples were turbid. Numerator = non-fecal counts; denominator = fecal counts.

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TABLE 9. FECAL AND TOTAL COLIFORMS IN SLUDGE SAMPLES FROM JAY ARC[a]

Date	Sludge Pond	Pensacola	Montclair	W. Fla. Util.
10/05/76	14,000[b]	28,000[b]	160,000[b]	-
11/02/76	400/0	154,000/11,200	78,000/26,000	20,300/12,000
12/07/76	18,000/10,000	54,000/40,000	240,000/ -	160,000/140,000
01/04/77	165,500[b]	3,500[b]	32,500[b]	50,000[b]
02/02/77	250,000/150,000	450,000/300,000	3,900,000/3,600,000	-
03/02/77	49,000/18,000	176,000/58,000	240,000/195,000	-
04/05/77	410,000/34,000	86,500/24,000	1,260,000/380,000	-
05/03/77	730,000/420,000	126,000/24,000	-	-
06/07/77	2,000/2,000	170,000/130,000	-	-
07/06/77	5,000/5,000	-	280,000/280,000	-
08/03/77	-	-	-	-
09/07/77	-	-	-	-
10/05/77	17,000/11,000	32,500/14,000	33,000/17,000	-
11/02/77	35,000/-	18,000/ -	44,000/ -	-
12/07/77	46,500/41,000	41,000/34,000	54,000/50,000	-

TABLE 9. Continued

Date	Sludge Pond	Pensacola	Montclair	W. Fla. Util.
01/06/78	8,000/7,000	70,000/26,000	79,000/13,000	----
02/10/78	-	-	33,000/16,300	----
04/03/78	3,500/2,200	-	5,400/1,600	----
05/03/78	140/40	16,000/5,400	>24,000/170	----
06/05/78	130/130	110/<20	16,000/16,000	----
07/10/78	170/<20	-/<20	330/130	----
08/09/78	490/20	230/20	>24,000/>24,000	----
09/11/78	790/320	1,600/920	-	----
10/04/78	22/0	>2,400/920	> 2,400/>2,400	----
11/01/78	20/20	9,200/1,100	>24,000/>24,000	----
12/06/78	>24,000/>24,000	2,564/20	>24,000/>24,000	----
01/10/79	>24,000/540	>24,000/>24,000	>24,000/>24,000	----
02/12/79	9,200/2,400	16,000/1,100	>24,000/>24,000	----
03/07/79	24,000/790	>24,000/>24,000	>24,000/>24,000	----
04/09/79	2,200/1,100	20/20	16,000/5,400	----

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TABLE 9. Continued

Date	Sludge Pond	Pensacola	Montclair	W. Fla. Util.
05/03/79	160,000/54,000	54,000/13,000	-	----
06/05/79	-	-	-	----
07/10/79	>24,000/9,200	>24,000/>24,000	>24,000/>24,000	----
08/07/79	>24,000/9,200	16,000/16,000	>2,400/70	----

[a] MPN assays per 100 ml. Numerator = total coliforms; denominator = fecal coliforms.

[b] Total coliforms only.

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TABLE 10. PRELIMINARY FEEDING TRIALS INITIATED JANUARY, 1976
JAY AND LIVE OAK, FLORIDA

Abbreviated Bacterial Analysis

Code	Wet/Dry	MPN *E. coli*/gram				Summary
		2-12-76	3-2-76	4-8-76	5-13-76	
Forage						
55E R-1						The most common organisms encountered in feed or forage were *Enterobacter* (several species), *Psuedomonas*, and *Citrobacter freundii*
51E R-2						
52E R-1						
61W R-2						
52W R-1						
55W R-2						
Feed						
1-29-76			0			No animal pathogens identified on 1-2-76, 2-12-76, 3-2-76, 4-8-76, or 5-13-76*
2-20-76			0			
1-29-76			0			
2-16-76			1,600			
2-11-76			0			
2-20-76			0			
2-26-76			0			
Forage						
55-W						
55-E						
52-W						
52-E						
61-W						
61-E						

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TABLE 10. Continued

Code	Wet/Dry	2-12-76	3-2-76	4-8-76	5-13-76	Summary
Sludge						
1-2-76	Wet	ND				Organisms most frequently encountered in sludge were *Enterobacter* (several species), *Escherichia coli*, and *Proteus* (several species) * No animal pathogens were identified (at above periods)
2-6-76	Dry	2,400				
2-6-76	Wet	11,000				
4-8-76	Wet			24,000		
	Wet			16,000		
	Wet			16,000		
	Dry			5,400		
5-13-76	Fresh/Wet				24,000	
	Fresh/Dry				16,000	
Soil-Jay						
55E R-1		-		-	600	The most common non-lactose fermenting organisms encountered in soil were *Proteus* and *Enterobacter*. *E. coli* counts are total coliforms and not fecal coliforms. They could be from the sludge or naturally occurring. * No animal pathogens were identified (at above periods)
61E R-2		-		-	282	
52E R-1		2,400		460	-	
61W R-2		-		-	285	
52W R-1		1,100		3,500	6,000	
55W R-2		-		5,400	2,300	

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FATE OF VIRUSES FOLLOWING APPLICATION OF MUNICIPAL SLUDGE TO LAND

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INTRODUCTION

In the U.S., the Water Pollution Control Act of 1972 (PL 92-500), as recently amended, requires acceptable methods for the utilization and disposal of sludge. It now appears that land disposal of sewage effluents and residuals is a viable and attractive alternative (Smith and Bryan, 1975; Sopper and Kardos, 1973; Thabaraj, 1975; Wright, 1975). Land spreading of wastewater effluents and residuals has many advantages, including the addition of plant nutrients, water conservation, improvement of soil physical properties, and increased soil organic matter. However, concern was raised over the contamination of surface and groundwater with microbial pathogens, particularly viruses (Bitton, 1975; Bitton, 1980; Burge and March, 1978; Cliver, 1976; Foster and Engelbrecht, 1973; Gerba *et al.*, 1975; Sagik and Sorber, 1978).

Viruses are generally associated with wastewater solids (Cliver, 1976; Duff, 1970; Lund, 1974; Moore *et al.*, 1976; Ward and Ashley, 1976; Wellings *et al.*, 1977) and are merely transferred to sludge as a result of wastewater treatment operations. Sludge treatment processes, such as anaerobic digestion, do not completely remove viruses (Bertucci *et al.*, 1977; Lund, 1974; Moore *et al.*, 1976).

Therefore, the application of anaerobically digested sludge onto land may lead to groundwater contamination as a result of virus transport through the soil matrix. The movement of sludge-associated viruses is probably limited due to the immobilization of sludge particles which have not become associated with the sludge solids or which dissociate from the solids as a result of changes in the physico-chemical properties within the soil matrix. The survival and transport of these infectious particles is of major concern in sludge application to land.

This work is divided into 4 phases. In the first phase we undertook laboratory experiments to gain some understanding of transport of sludge-associated viruses through Florida soils. In the second phase of the project, we evaluated the methodology for virus detection in soils and sludges - a new method was developed for virus detection in soils. The selected methods were used in phases 3 and 4. In the third phase we were concerned with the survival and transport of enteroviruses through soil cores exposed

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to natural conditions. The major environmental parameters (soil temperature, rainfall, soil moisture) were monitored at the experimental site. Finally, the fourth phase involved the monitoring of sludges, soils, and groundwater at 2 sludge disposal sites in Florida.

MATERIALS AND METHODS

Viruses and Viral Assays

Poliovirus type 1 (Sabin) and echovirus type 1 (Farouk) were used in this phase of the project. Stocks were prepared as previously described (Bitton *et al.*, 1976). The viruses were kept at -70°C until used.

The viruses were concentrated prior to use in the soil column experiments by either ultracentrifugation or by the method developed by Farrah *et al.* (1978) which involved blending with trichlorotrifluoroethane (Freon 113, DuPont DeNemouns Co., Wilmington, DL) followed by concentration on Filterite filters.

Assay was by the plaque technique on AV3 (human amnion) or MA104 (simian kidney) cell monolayers grown in Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum (FCS), 250 units (U) of penicillin per ml, and 125 μg of streptomycin per ml. Each experiment was assayed completely on only one cell line (AV3 or MA104) using the procedure previously described (Bitton *et al.*, 1976). If necessary, samples were diluted prior to assay in phosphate-buffered saline (PBS) containing 2% FCS, 250 U of penicillin per ml, and 125 μg of streptomycin per ml. The numbers of viruses were expressed as plaque-forming units (PFU).

Virus Transport Through Soils: Laboratory Experiments

The procedures described by Bitton *et al.* (1978) have been presented at the International Water Pollution Conference in Stockholm, Sweden.

Development of Methodology for Virus Recovery from Soils and Sludges

Virus recovery from soils--The procedures have been described in detail by Bitton *et al.* (1979).

Virus recovery from sludges--A modified version of the procedure of Hurst *et al.* (2978) was used for virus recovery from sludges. This method is described elsewhere (see "Virus monitoring in sludge application sites").

Survival and Transport of Viruses Following Sludge Application to Columns Exposed to Natural Conditions

The sludge used was a lagooned sludge sampled at the West Florida Agricultural Experiment Station (Jay, Florida). This sludge was a mixture of aerobically digested sludge (1/3) and anaerobically digested sludge (2/3) from the Montclair and Main Street wastewater treatment plants of Pensacola, Florida, respectively. The mixture was kept in a lagoon at the West Florida

Agricultural Experiment Station before ultimately being spread on land. It was the lagooned sludge which was sampled and used in this study.

Sludge from the Main Street, Kanapaha site, wastewater treatment plant of Gainesville, Florida, was also used in one experiment. The sludge used was aerobically digested for 180 days.

The sludges were collected in sterile Nalgene bottles, transported to the University of Florida (Gainesville) laboratory and then immediately refrigerated. At the time of use, a sludge sample was first allowed to come to room temperature. The pH and solid content of the sludge was then determined. The pH was measured using a Beckman Expandomatic SS-2 pH meter. The solids content was determined by drying a measured volume of sludge in an oven at 105^{o}C for 24 hours and expressed as a percentage on a weight (grams) to volume (milliliters) basis.

The soil used was a Eustis fine sand sampled at the agronomy farm, University of Florida. Gainesville. This was classified as a Psammentic Paleudult, sandy siliceous, hyperthermic soil (Calhoun *et al.*, 1974).

Undisturbed cores of this soil were obtained by driving polyvinyl chloride pipes (40 cm in length and 5 cm or 15.5 cm inside diameter) 33 cm into the soil; thereby consisting of the Ap and A21 soil horizons. The soil cores were placed outside the Environmental Engineering Sciences building at the University of Florida under natural conditions. The soil cores rested on a wooden box such that soil leachates following rainfall could be collected (see Figure 1). The large soil cores were insulated by surrounding them with duct insulation. Porous ceramic cups were installed at the bottom of the large cores in order to facilitate sampling of the soil water during periods of low rainfall. These cups were evaluated for their retentive capacity toward viruses as described below.

The soil temperature, soil moisture, and rainfall were monitored. The soil temperature was monitored every hour using thermocouples placed at the soil surface and at depths of 2.5, 10, and 20 cm on one of the large soil cores. The thermocouples were connected to an Esterline Angus Key Programmable Data Acquisition System (model PD-2064, Esterline Angus Instrument Corporation, Indianapolis, IN) which printed voltage (millivolts) at each thermocouple every hour. The voltages measured were later converted to temperature readings with the use of a computer. The soil moisture was determined by drying (a measured weight of wet soil from the top inch of the soil cores) in an oven at 105^{o}C for 24 hours and was expressed as a percentage. The rainfall was measured next to the soil columns with a farm rain gage (number 510, Science Associates Inc., Princeton, NJ) attached to the wooden box. The rainfall was measured after each rain event in centimeters.

Poliovirus type 1 (Sabin) or echovirus type 1 (Farouk) was seeded in liquid sludge and mixed for 10 minutes to 1 hour using a magnetic stirrer. The sludge was assayed for virus directly (i.e., without solids separation). The supernatant following centrifugation (12,000 rpm or 20,842 x g for 30 min at 4^{o}C) was also assayed. This allowed the determination of the fraction of viruses associated with sludge solids before the application of the sludge to the soil columns. Following the adsorption period and the initial viral assays, 2.5 cm of the virus-seeded sludge was applied to the soil columns. Sludge seeded with echovirus was applied to 2 large soil cores (15.5 cm inside diameter). The sludge, seeded with poliovirus, was applied similarly to a separate set of soil cores. The seeded-sludge was allowed to soak in and dry on top of the soil for 3 to 4 days before being mixed with the top 2.5 cm of soil. Virus monitoring of the drying sludge solids on top of the soil was undertaken as described below. The soil was also monitored for viruses after the sludge was worked under 2.5 cm. The top 2.5 cm of soil was monitored using the methods described elsewhere.

Virus Monitoring in Sludge Application Sites

Virus recovery from sludge--A modified version of the procedure of Hurst *et al.* (1978) was used to recover viruses from sludge. The modified version was described in detail by Pancorbo *et al.* (Canadian Journal of Microbiology, in press).

Lagoon water--Four liters of water overlying the lagooned sludge was adjusted to pH 2.5 by addition of 1 M, pH 2 glycine and centrifuged at 15,000 x g for 10 min. The supernatant was passed through a series of 2 0.45 μm Filterite filters in 47 mm holders. Seven ml of PBS + 10% FCS, pH 9, was passed through the filters, neutralized and saved as the final sample. The pellet remaining after centrifugation was mixed with an equal volume of PBS + 10% FCS, pH 9, readjusted to pH 9 by addition of 1 M, pH 11.5 glycine, and centrifuged at 15,000 x g for 10 min. The supernatant was removed and neutralized by addition of 0.05 M, pH 2 glycine before assay.

Virus concentration procedure for well water--Water from two wells was hand pumped into 100 gallon tanks. The water was adjusted to pH 3.5 by addition of 0.2 N HCl and adjusted to 0.0005 M aluminum chloride. Water from the other wells was removed using deep-well pumps, added to 100 gallon tanks and adjusted as described above or was adjusted to pH 3.5 and 0.0005 M aluminum chloride by in-line injection of acid and salts (Gerba *et al.*, 1978). The treated water was passed through a 10 inch, 0.45 μm porosity Filterite filter (Filterite Corp., Timonium, MD). The filters were then treated with 800 ml of 0.05 M glycine, pH 11.5. The glycine solution was permitted to remain in contact with the filters for one minute, removed, and neutralized by addition of 1 M, pH 2 glycine. The neutralized samples were stored on ice for 24 to 48 hours during transportation to the laboratory. The samples were then adjusted to pH 3.5 by addition of 1 M pH 2 glycine and centrifuged at 800 x g for 10 min. The supernatants were passed through a series of 2 0.45 μm Filterite filters in 47 mm holders. Next, 7 ml of 0.05 M glycine + 2% FCS was passed through the filters, neutralized, and used as the inoculum for cell cultures. The sediment remaining after centrifugation at pH 3.5 was mixed with 5 volumes of phosphate buffered saline (PBS) with 10% FCS at pH 9, readjusted

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to pH 9 by addition of 1 M, pH 11.5 glycine, if necessary. After centrifugation at 15,000 xg for 10 minutes, the supernatant was removed, neutralized, and used for inoculation of cell cultures.

Virus recovery from soils--The methods of Hurst and Gerba (1979) and of Bitton *et al.* (1979) were used to recover virus from soils at the Kanapaha site and Jay site, respectively.

Weather data for the Jay site--Weather data were supplied by the West Florida Agricultural Experiment Station at Jay. Mean monthly air temperatures (maximum and minimum) and total monthly precipitation from September 1977 through March 1979 are shown in Figure 2.

Cell cultures and viral assays--BGM (Baron *et al.*, 1970) or primary monkey kidney cells (Flow Laboratories, Inc., McLean, VA) were used for isolation of viruses. Serial dilutions of samples were made in PBS + 2% FCS and used to inoculate cell cultures. The cells were examined for cytopathic effects (CPE) for up to 3 weeks. Samples showing CPE were frozen, thawed, diluted 1/100 to 1/10,000 and used to reinoculate cell cultures. Samples that produced CPE on the second passage were frozen. The titer of these isolates was determined using MA-104 cells with a methyl-cellulose overlay. Neutralization with specific antisera was used to identify isolates (Lim and Benyesh-Melnick, 1960). Tissue-culture infective dose ($TCID_{50}$) was determined according to the method of Reed and Muench (1938).

Ultracentrifugation--Samples with excessive final volumes were centrifuged at 120,000 x g for 90 minutes in a TI-60 rotor using a Beckman Model L3-50 ultracentrifuge (Beckman Instruments, Fullerton, CA). The pellets were suspended in FCS and assayed.

RESULTS AND DISCUSSION

Virus Transport Through Soils: Laboratory Experiments

Prior to conducting field experiments, it appeared necessary to study the pattern of virus transport through the soil under consideration, Red Bay sandy loam. This soil has been sampled at the Jay site. Laboratory packed soil columns and undisturbed soil cores were employed. The results showed that the soil under study retained more than 99% of poliovirus type 1 following sludge application. The mechanisms involved in the transport of viruses in sludge-soil systems are discussed by Bitton *et al.* (1978) and the results have been presented at the International Water Pollution Conference in Stockholm, Sweden.

Development of Methodology for Virus Recovery from Soils and Sludges

Virus recovery from soils--Elution of poliovirus type 1 from Eustis fine sand--The efficiency of virus elution from a sandy soil was examined for 3% beef extract, tryptose phosphate broth, 10% FCS in PBS, a muck solution, 0.25 M glycine in combination with 0.05 M EDTA, 0.25 M glycine, 0.2% purified casein, 0.5% isoelectric casein, and 0.5% suspension of nonfat dry milk.

Elution of poliovirus from soil ranged from 15% with the muck solution to more than 100% with 0.5% isoelectric casein at pH 9. Glycine-EDTA, at a pH of 11.5 (the pH dropped to 11 upon mixing with soil), displayed 61% elution and similar efficiency was achieved with 0.25 M glycine alone (55%). The elution efficiency was significantly higher with purified casein, isoelectric casein, and non-fat dry milk than with glycine-EDTA or beef extract. Further concentration of soil eluates can be achieved with 7 out of 9 eluents examined. Four eluents (beef extract, glycine-EDTA, isoelectric casein, and non-fat dry milk) were further examined for the recovery efficiency of the concentration step.

The overall recovery (elution followed by a concentration step) of poliovirus type 1 (Sabin) from soil was examined with regards to beef extract, 0.25 M glycine + 0.05 M EDTA, 0.5% isoelectric casein, and 0.5% non-fat dry milk. The elution was performed at pH 9 for casein, non-fat dry milk, and beef extract, and at pH 11 for glycine-EDTA. The results are shown in Table 1. The best eluents were 0.5% isoelectric casein and 0.5% non-fat dry milk. However, beef extract performed best during the concentration step (organic flocculation). The overall recovery of poliovirus was 38%, 41%, 60%, and 66% with beef extract, glycine-EDTA, isoelectric casein, and non-fat dry milk, respectively. Analysis of variance did not show any significant difference at the 0.05 level, among the 4 methods for overall virus recovery. Isoelectric casein was selected to further study the recovery of poliovirus type 1 from 4 soil types as well as that of other enteroviruses. It was found that the recovery of poliovirus varied from 45% to 52% for the 4 soils under study (Table 2). Analysis of variance did not show any significant difference between the 4 soil types.

Overall recovery of poliovirus 1, coxsackievirus B3, and echovirus 4 by the casein method is displayed in Table 3. It was shown that virus elution was generally high and ranged from 73 to more than 100%. Echovirus 4 was, however, not well recovered during the concentration step and the overall recovery was only 23%. With coxsackievirus B3, the overall recovery was significantly higher (at the 0.05 level) than poliovirus type 1 or echovirus 4.

Four methods have been described for virus recovery from Eustis fine sand, which is representative of soils found in Florida. An ideal method should include an efficient elution step followed by a concentration step which would aid in the detection of low numbers of viruses in relatively large amounts (100-200 g) of soil. Regarding the elution step, the purpose was to select an eluent that would operate efficiently at pH 9 and that would be easily amenable to further concentration. Among 9 eluents tested, 0.5% isoelectric casein and 0.5% non-fat dry milk were the most efficient ones in desorbing viruses from soil. These eluents operate at pH 9 and thus avoid the potential harmful effect of high pHs (pH 11-11.5) toward enteroviruses. Exposure over 10 min to glycine buffer at pH 11.5 may also be harmful to poliovirus type 1 (Gerba *et al.*, 1977). Another advantage of these eluents was allowing further concentration of viruses by organic flocculation. This step was similar to that observed with beef extract (Katzenelson *et al.*, 1976) except that flocculation occurs

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at pH 4.5 (Jenness and Patton, 1959) instead of pH 3.5. With regard to overall virus recovery, no significant differences were shown between beef extract, isoelectric casein, non-fat dry milk, and glycine-EDTA methods.

The isoelectric method was able to recover high (4.5×10^6 - 9.3×10^6) and low (2×10^2) inputs of viruses from 100 g of soil with efficiencies of 50% and 75%, respectively. These recovery efficiencied are similar to those reported by Gerba *et al.* (1977) with respect to virus recovery from estuarine sediments. These investigators have used 0.25 M glycine - 0.05 M EDTA (pH 11) as eluting solution and reported a mean recovery of 50%. Glycine-EDTA has been shown to be effective for recovering poliovirus 4 as well as poliovirus 1 or coxsackievirus B3. It appears (Table 3) that echovirus 4 was efficiently eluted from soil particles (75% elution efficiency) but it was not well recovered (30%) during the concentration step. Research is now being undertaken to improve the concentration step. It also appears that the recovery efficiency of the casein method is not significantly affected by soil type and a 50% mean recovery was achieved with 4 Florida soils.

Methodology for Virus Recovery from Sludges

Virus recovery from sludges was performed according to a modification of the method developed by Hurst *et al.* (1978). The use of this method for mixed liquor and anaerobically digested sludge resulted in recovery efficiencies similar to those reported by Hurst *et al.* (1978). However, the use of this method for aerobically digested sludge resulted in poor recovery. Therefore, a series of experiments were undertaken to study the effect of sludge type on virus recovery by the Hurst *et al.*, method.

Virus association with sludge solids was studied, using mixed liquor and wasted, aerobic and anaerobic sludges (data not shown). It was found that the percent association of viruses with sludge solids was significantly higher (at the 0.01 level using Duncan's test) for wasted and aerobic sludges than for mixed liquor and anaerobic sludge. In seeded experiments using poliovirus type 1, virus recovery was much higher for mixed liquor and anaerobic sludge than for wasted and aerobic sludge (Table 4). For anaerobically digested sludge, the mean percent recovery was 60.2% whereas for aerobically digested sludge the mean recovery was 14.5%. The difference in percent recovery was significiant at the 0.01 level, using Duncan's test. A relatively high recovery (72.3%) was obtained, using mixed liquor. This probably is the type of material used by Hurst *et al.* (1978) with regard to the development of their method.

These findings are significant since many types of sludge have been used in this research project; hopefully, a high recovery was achieved with the lagoon sludge from the Jay site. This particular sludge is predominantly anaerobic and thus allows a relatively good virus recovery.

It is thus necessary to take in consideration the sludge type when one contemplates the development of methods for virus recovery from sludge.

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Survival and Transport of Viruses Following Sludge Application to Soil Columns Exposed to Natural Conditions

In previous sections, virus transport through soils under controlled laboratory conditions has been described as adequate method for virus detection in soils and sludges. It thus appeared necessary to study virus transport and survival under more natural conditions. Undisturbed soil cores (33 cm long and 15.5 cm inside diameter) were used to assess virus transport and survival under field conditions. In these experiments, environmental parameters (temperature, soil moisture, rainfall) were monitored; more details are given in the "Materials and Methods" section. The protocol of sludge disposal to soil was similar to that practiced in sludge disposal sites.

Virus survival and transport were monitored during the summer and fall season, using the same columns. Survival monitoring was terminated when viruses were not detectable in soil samples.

Summer season--This period is generally warm and wet in the Gainesville area. Soil temperature was measured with thermocouples placed at the surface, 2.5, 10, and 20 cm depth. Data analysis showed that there was no significant difference between temperature readings at these depths. The average temperature ranged from 23.5°C to 29°C during the 35-day period.

The study period was very wet with 13.6 cm of rainfall from June 2 to July 7, 1978. Poliovirus survival was monitored in 2 soil cores which had been treated with seeded sludge (Table 5). There was some decline in virus numbers in the sludge prior to mixing with the top 2.5 cm of soil. Soil monitoring revealed that poliovirus could be detected up to 35 days in both cores. It is difficult to correlate virus survival to soil moisture since this parameter was not continuously monitored. Heavy rainfall did not allow the soil to dry for an extended period of time and this probably has led to prolonged virus survival.

Monitoring of soil leachates from June 5 to August 24, 1978, did not reveal any virus, despite their concentration by membrane filtration (Table 6). Although 51 cm of rain fell during the study period, this represented only 0.5 to 0.7 pore volume. It appears that viruses are efficiently retained by soils under unsaturated flow conditions.

Fall season--Sludge application was undertaken again on October 11, 1979, and virus presence in the soil and leachates was monitored for 21 and 101 days, respectively.

It became apparent that with regard to transport pattern, poliovirus type 1 (Sabin) would not be the ideal model virus since it has a high affinity for sludge solids and is subsequently immobilized at the top layer of soil. A virus with less affinity for sludge solids would be more suitable for transport studies. In other studies, echovirus 1 (Farouk) was found to be less adsorbed to soil than poliovirus type 1 (Sabin). The association between lagooned sludge solids (sludge used at the sludge disposal site in Jay, Florida) and poliovirus type 1 and echovirus was therefore studied (Table 7). It was found that echovirus 1 was less adsorbed (21.7%) to sludge solids than poliovirus type 1 (95.2%). Lagooned sludge from Jay was seeded with either of these 2 enteroviruses and then applied to the soil cores.

During the study period the average temperature, monitored with thermocouples placed in soil cores, ranged from $18^{o}C$ to $27^{o}C$. Only 0.13 cm of rain fell from October 11, 1978 to November 1, 1978. This was the period during which virus survival was monitored. Neither virus could be detected in soil after 21 days of incubation under natural conditions. The 2 enteroviruses were completely inactivated sometime between day 8 and 21 (Table 8).

Soil leachates were also monitored and a summary of the data is displayed in Table 9. Neither poliovirus nor echovirus was detected in the leachates from all the soil cores (Table 9).

It appears from these studies that, under conditions prevailing in North Central Florida, enteroviruses are rapidly inactivated during sludge application to soils. Their inactivation in the soil appears to be affected more by desiccation than by soil temperature. Under ideal conditions (warm and dry), a rapid decline of virus was observed in the sludge drying on top of the soil. Soil leachates collected after natural rainfall were negative for both poliovirus type 1 (Sabin) and echovirus 1 (Farouk).

Virus Monitoring at Two Sludge Disposal Sites

Two sludge disposal sites were selected for virus monitoring: Kanapaha near Gainesville, Florida, and Jay site near Pensacola, Florida. The monitoring involved attempts to recover viruses from groundwater, sludge, and soil samples from the disposal sites.

Virus monitoring at the Kanapaha sludge disposal site--During 3 consecutive months (December, 1977; January, 1978; and February, 1978), the City of Gainesville sludge disposal site (10 acres) adjacent to Lake Kanapaha, Florida, was monitored for viruses. Aerobically digested sludge (180 days detention time) conditioned with a cationic polymer and dewatered by centrifugation was applied to this site and immediately incorporated into the soil (Figure 3). The groundwater, soil, and centrifuged sludge was monitored for indigenous viruses. The centrifuged sludge applied at the time this study was performed originated at the Main Street treatment plant of Gainesville. Since the conclusion of our study, the sludge from the Kanapaha treatment plant has also been disposed of at this site. The sludge is transported to the site in tank trucks and is spread out onto the soil. The sludge is disked into the soil as soon as it is applied except when a cover crop is present. In the presence of a cover crop, the sludge is applied as a top dressing on the crop. Coastal bermudagrass is utilized during the summer months while ryegrass is in the winter months (Gainesville-Alachua County Regional Utilities, 1976). The monitoring wells located throughout the sludge disposal site are 60 feet in depth. The groundwater flows in a northwesterly direction as shown in Figure 4. The soil found at the site belongs to the Lochloosa series (Gainesville-Alachua County Regional Utilities, 1976). This series is a member of the loamy,

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siliceous, hyperthermic family of Aquic Arenic Paleudults. These soils have thick sandy A horizons; mottled light yellowish brown B1 horizons; yellowish brown B21t horizons; and mottled gray B22tg and B3g horizons. The typifying pedon is Lochloosa fine sand. The methodology for virus recovery from well water, sludge, and soil is described in the "Materials and Methods" section.

The results obtained are shown in Table 10. Enteroviruses were recovered from waste sludge but were inactivated almost to undetectable levels after 90 days detention in an aerobic digestor. The sludge ultimately disposed of at the Kanapaha site was aerobically digested for 180 days, conditioned with a cationic polymer and dewatered by centrifugation. Enteroviruses could not be recovered from the centrifuged sludge. Thus, it is understandable that no viruses were detected in the soil and groundwater at the Kanapaha site. By increasing the detention time of the sludge at the wastewater treatment plant, the viral hazards of sludge disposal on land were probably eliminated. Therefore, our efforts were concentrated on virus monitoring at the Jay site.

Agricultural Research Center at Jay, Florida--Sludge originating from Pensacola, Florida, has been applied for some years on 10 acres at the IFAS Agricultural Research Center in Jay, Florida. The sludge contained 3-4% solids and was lagooned prior to land application at a rate of . A virus monitoring program has been undertaken to assess the virus load of sludge, soil, and groundwater at the Jay site. Moreover, we investigated the survival of indigenous viruses in the sludge lagoon.

a. Sludge monitoring--The sludge used in Jay originated from 3 wastewater treatment plants, located in Pensacola, Florida. Two-thirds of the sludge was anaerobically digested (Main Street and Northeast plants) and one-third was aerobically digested (Montclair plant). Despite the poor performance of the Hurst *et al.*, method with aerobic sludge, the virus load of aerobically digested sludge was always higher than that of anaerobically digested sludge (Table 11). These findings raise concern over the efficiency of aerobic digestion with regard to virus destruction.

b. Survival of enteroviruses in the sludge lagoon--The sludges were transported by tank trucks from Pensacola to a sludge lagoon located at the Jay site. The lagoon was approximately 60 x 100 ft, containing liquid sludge to a depth of approximately 6 ft, and contained approximately 1 million gallons of liquid sludge. Sludge addition to and removal from the lagoon were carried out at the opposite ends of the lagoon. At certain stages of crop growth, land applications of sludge as well as the addition of digested sludges to the lagoon were suspended. This practice provided a unique opportunity to study the fate of indigenous enteroviruses in the sludge lagoon. Enteroviruses were readily recovered from the sludge (15 and 80 TCID 50/g of sludge) during periods when digested sludge was added to the lagoon. The addition of fresh digested sludge to the lagoon was suspended on April 14, 1978. For the next 6 months, viruses were found in very low numbers or were undetected in samples of lagooned sludge. They were again detected when the addition of digested sludge to the lagoon was resumed on November 7, 1978 (Figure 6).

In order to determine if viruses were being eluted from the lagooned sludge solids and then transferred to the overlying water, samples of lagooned water were obtained while sludge addition to the lagoon was suspended and after it was resumed. Enteroviruses were found in the water when sludge was being added to the lagoon and were not found when sludge addition was suspended. The lagooned water had a large number of algae as shown by the concentration of chlorophyll a (Table 12).

Polioviruses (type 1, 2, and 3), coxsackie B viruses (B4), and echoviruses (type 1, 7, and 15) were isolated from sludge and overlying water samples taken from the lagoon (Table 13). A variation in the relative number of isolates was observed. Polioviruses represented greater than 90% of the isolates obtained from samples collected from 2-17 to 11-6-78. Polio-1 was the most common serotype isolated from these samples. From 12-6-78 to 1-24-79, poliovirus serotypes represented less than 50% of the isolates obtained while greater numbers of echo and coxsackie B viruses were recovered (Table 13).

These data (Figure 6) show that sludge lagooning may be an efficient means of reducing viral numbers prior to land application under the warm climate of Florida. No information could be found in the literature with regard to virus survival in sludge lagoons.

Indigenous virus survival in lagoon sludge freshly applied to land was monitored on October 3, 1979. Virus was detected in sludge; using 2 methods, glycine and lysine. Table 14 shows that enteroviruses could be detected for up to 9 days in sludge prior to mixing with soil.

c. Groundwater monitoring--Monitoring wells were dug in the study site and their position is shown in figure 7. Their depth was 70 to 80 feet. Two other wells (not shown in figure 7) were dug at 10 ft from the sludge lagoon. All the wells were monitored at bimonthly intervals for a period of one year. The results have been summarized in Table 15. No virus has been detected in any of the groundwater samples.

Previous studies have dealt with virus transport through soils treated with sewage effluents (Bitton *et al.*, 1976; Bitton *et al.*, 1979; Duboise *et al.*, 1976; Gilbert *et al.*, 1976; Goyal and Gerba, 1979; Lance and Gerba, 1977; Lance *et al.*, 1976; Landry *et al.*, 1979; Schaub and Sorber, 1977; Scheuerman *et al.*, 1979). However, few studies have dealt with virus transport in sludge-amended soils (Damgaard-Larsen *et al.*, 1977; Moore *et al.*, 1978). Damgaard-Larsen *et al.*, used lysimeters to study virus transport in sludge-amended soils. Viruses were completely retained by the soils under study. Moore *et al.* (1978) investigated virus survival in sludge-amended soils but no groundwater monitoring was reported.

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d. Soil monitoring--Following storage in the lagoon, the sludge was ultimately spread on land. The sludge was allowed to dry on top of the soil for 2 to 14 days, and then mixed with the soil. The field used for sludge disposal (Figure 7) was divided into 72 plots of 40 x 120 ft, which received from 0 to 15 acre-inches of sludge per year. Plots numbered 1, 32, and 61 which received 15 acre-inches of sludge per year were monitored for viruses from June, 1978 through January, 1979. In addition, the plot numbered 42, which received no sludge, was monitored as a control.

We have shown that enteroviruses were readily recovered from sludges, including lagooned sludge (Table 13). However, no virus could be detected in soil samples at the Jay site. Allowing the sludge to dry on top of the soil before being mixed with the soil results in the inactivation of all or most of the enteroviruses present. This may be an advantage over sludge injection into soils (Moore *et al.*, 1978), where viruses can survive for longer periods of time. Despite the numerous advantages of sludge injection (aesthetic acceptability, odor and runoff are minimized), surface spreading of sludge may result in higher inactivation of viruses.

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TABLE 1. OVERALL RECOVERY OF POLIOVIRUS TYPE 1 (SABIN) FROM 10 G OF SOIL (EUSTIS FINE SAND), USING 4 DIFFERENT METHODS

Recovery method	Total virus input (PFU)[1]	Percent elution[2]	Step[3] Conc. % Recovery	Overall recovery percent	Mean overall recovery (% ± SE)
3% Beef Extract (pH = 9.0)	5.0×10^5	41	95	39	37.7 ± 6.5[4]
		52	56	29	
		52	86	45	
0.25 M Glycine + 0.05 M EDTA (pH = 11.0)	3.8×10^5	77	61	47	41.0 ± 8
		48	72	35	
0.5% isoelectric casein (pH = 9.0)	4.7×10^5	128	40	51	59.5 ± 8
		123	55	68	
0.5% non-fat dry milk (pH = 9.0)	6.8×10^5	98	80	78	66.0 ± 8
		92	59	54	

---

[1] Ten pore volumes of autoclaved secondary sewage effluent seeded with virus and passed through 10 g soil columns. Two pore volumes of non-seeded secondary sewage effluent passed through the columns to leach out non-associated viruses. Soil leachates assayed to determine viruses retained by 10 g of soil.

[2] 30 ml of eluents added to 10 g soil samples. Samples vortexed for 30 sec, shaken for 15 min (in the presence of glycine or glycine-EDTA, the shaking time was 4.5 min), centrifuged at 4000 rpm for 4 min. Supernatants decanted and 5 ml of aliquot of supernatants brought to pH = 7.0-7.5 with 1 M glycine (pH = 1.3-1.8). Samples made isotonic and sometimes diluted in MEM prior to assay.

TABLE 1. Continued

[3] Eluates concentrated by organic flocculation at pH 3.5 for beef extract; 4.5 for isoelectric casein and non-fat dry milk. Glycine-EDTA eluates were flocculated at pH 3.5 with 0.06 M AlCl . Flocs eluted with fetal calf serum in PBS at pH 9.0. Supernatants concentrated by membrane filtration.

[4] ANOVA. No significant difference at the 0.05 level among 4 methods.

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TABLE 2. RECOVERY OF POLIOVIRUS TYPE 1 (SABIN) FROM 4 SOILS (100 G) BY THE ISOELECTRIC CASEIN METHOD

	Input (PFU)[1]	Percent[2]	Recovery (% ± SE)[3]
Eustis Fine Sand	3.5×10^{6}	49 55	52.0 ± 8
Jay (Top Soil)	1.6×10^{5}	37 60	48.5 ± 8
Cypress Dome Sand	7.0×10^{4}	39 60	49.5 ± 8
Red Bay Sandy Loan	1.5×10^{5}	46 44	45.0 ± 8

[1] Ten pore volumes secondary sewage effluent seeded with virus passed through 100 g soil columns; 2 pore volumes sterile secondary sewage effluent passed to ensure adsorption. Leachates assayed to determine amount of virus retained by soil.

[2] 0.5% isoelectric casein (200 ml) pH 9.0 added to soil samples. Samples were shaken 15 min, centrifuged at 10,000 rpm for 4 min. Supernatant was decanted and centrifuged at 10,000 rpm for 4 min, brought to pH = 4.5 with 1 M glycine (pH = 2.0). Resulting floc centrifuged at 2,000 rpm for 2 min. Supernatant decanted and remaining pellet was resuspended in 0.15 M Na_2HPO_4 at pH = 9.0.

[3] ANOVA. No significant difference at the 0.05 level among the 4 soils.

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TABLE 3. RECOVERY OF 3 ENTEROVIRUSES FROM SOIL (EUSTIS FINE SAND) WITH 0.5% (W/V) ISOELECTRIC CASEIN AT pH 9.0

Virus	Virus input PFU/10 g Soil[1]	% Elution[2]	Step[3] Conc % Recovery	Overall Recovery %	Mean overall recovery (% ± SE)
Poliovirus 1	4.7×10^5	128	40	51	59.5 ± 5.5b[4]
		123	55	68	
Coxsackie B3	1.6×10^5	125	74	92	88 ± 5.5a
		111	76	81	
Echo 4	1.9×10^5	76	32	24	22.5 ± 5.5c
		73	28	21	

[1] Ten pore volumes autoclaved secondary sewage effluent seeded with virus and passed through 10 g soil columns. Two pore volumes of non-seeded secondary sewage effluent passed through the soil columns to leach out non-associated viruses. Soil leachates assayed to determine viruses retained by 10 g of soil.

[2] 30 ml of the eluents were added to 10 g soil samples. Samples were vortexed for 30 sec, shaken for 15 min, and centrifuged at 4,000 rpm for 4 min. Supernatants were decanted and 5 ml aliquot of the supernatants taken and brought to pH 7.0-7.5 with 1 M glycine (pH 1.3-1.8). Samples were made isotonic and sometimes diluted in MEM prior to assay.

[3] Soil eluates concentrated by organic flocculation at pH 4.5. Following centifugation, flocs were resuspended in 0.15 M Na_2HPO_4 at pH 9.0.

[4] Figures followed by similar letters did not differ significantly at the 0.05 level.

TABLE 4. EFFECT OF SLUDGE TYPE ON RECOVERY OF POLIOVIRUS FROM SLUDGE USING MODIFICATION OF HURST *ET AL.*, METHODS

Sludge type	Sludge used	Sludge parameters: pH	Sludge parameters: solids content (%)	Volume of sludge processed	Virus[b] added to sludge (total PFU)	Overall virus recovery[c] (%)	Mean[d] virus recovery for each sludge type (% ± SE)
Mixed liquor	UML	6.4	1.6	100	8.4 x 10	84.3	72.3 ± 12.0[a]
	GML	6.9	0.5	1,000	1.3 x 10	60.3	
Wasted	GW	6.2	2.4	1,000	7.4 x 10	8.3	--
Aerobically digested	UDA	4.8	1.5	500	6.5 x 10	18.9	14.5 ± 2.7[b]
	UDA	4.8	1.5	500	6.5 x 10	11.9	
	UDA	6.1	1.3	500	2.0 x 10	8.0	
	UDA	6.1	1.3	500	2.0 x 10	5.9	
	UDA	6.5	1.3	1,000	9.6 x 10	15.5	
	GDA90	5.8	1.0	1,000	1.3 x 10	14.1	
	PDA	5.8	2.8	1,000	4.1 x 10	26.9	
Anaerobically digested	PDAN	7.2	1.4	100	6.0 x 10	59.9	60.2 ± 2.1[a]
	PDAN	6.4	1.9	1,000	5.5 x 10	63.9	
	LAG[e]	7.3	2.9	1,000	1.8 x 10	56.7	

---

[a] Procedure - Hurst *et al.* (1978) and is described in the "Materials and Methods" section.

[b] Virus was added to sludge while stirring with magnetic stirrer. Stirring continued for 10 min-60 min; total sludge volume centrifuged at 1465 x g for 20 min at 4°C. Sludge supernatant and solids generated separately subjected to virus recovery methodology.

[c] Overall virus recovery (%) values determined from viruses recovered in final concentrates (sludge supernatant and sludge solids) were based on amount of virus (total PFU) added to sludge as 100%.

TABLE 4. Continued

[d] Mean values with different superscripts are significantly different at 0.01 level (ANOVA).

[e] Lagoon sludge is mixture of aerobically digested sludge (1/3), anaerobically digested sludge (2/3); consequently, its properties are close to those of anaerobically digested sludge. Therefore, lagoon sludges were placed in the population of anaerobically digested sludge.

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TABLE 5. SURVIVAL OF POLIOVIRUS TYPE 1 (SABIN), UNDER NATURAL CONDITIONS, FOLLOWING SUSPENSION IN LIQUID SLUDGE AND SUBSEQUENT APPLICATION TO LARGE SOIL CORES OF A EUSTIS FINE SAND[a] (June 2, 1978 - July 7, 1978)

Date	Days after beginning exper.	Cumulative rainfall[b] (cm)	Sludge solids (% w/v)	Soil moisture (% w/v)	Number viruses[c] (PFU/g dw of sludge or soil) Core 1	Core 2
Liquid Sludge Sample						
06-02-78	0	0	2.9	-[e]	5.6×10^7	5.6×10^7
Drying Sludge Samples						
06-03-78	1	1.80	23.1	-	2.5×10^6	4.9×10^6
	4	6.03	-	-	1.0×10^6	5.0×10^5
Sludge mixed with top 2.5 cm of soil on day 4						
Soil samples (top 2.5 cm)						
06-06-78	4	6.03	-	14.7	3.2×10^4	4.1×10^4
06-07-78	5	7.03	-	0.9	8.6×10^4	7.5×10^4
06-09-78	7	7.28	-	9.9	2.5×10^3	2.4×10^3
06-23-78	21	10.45	-	0.2	4.6	15.1
07-07-78	35	13.63	-	0.2	1.3	0.9

---

[a] 2.5 cm of lagooned sludge (sampled at West Florida Agricultural Experiment Station, Jay; sludge had pH of 6.9 and solids content of 2.9%) seeded with total of 7.8×10^8 PFU poliovirus applied on top of 33 cm undisturbed, large soil cores of Eustis fine sand (15.5 cm inside diameter; contains Ap and A21 horizons). Seeded sludge was allowed to soak in and dry on top of soil 4 days before being mixed with top 2.5 cm of soil.

[b] Rainfall data was collected at the experimental site.

[c] Virus monitoring of drying sludge undertaken as per "Materials and Methods" section which used 0.05 M glycine, pH 11.5 as primary eluent, and no further concentration of eluates. Virus monitoring of soil was undertaken by isoelectric casein method described in the "Materials and Methods" section.

TABLE 5. Continued

[d] Liquid sludge was seeded with 5.6 x 10 PFU of poliovirus per gram dry weight of sludge. A large fraction of the viruses seeded (91.5%) were associated with sludge solids after magnetic stirring for 1 hour and before application to the soil cores. Soil leachates from all cores were also monitored for viruses.

[e] - = not done

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TABLE 6. ANALYSIS FOR PRESENCE OF POLIOVIRUS TYPE 1 (SABIN) IN SOIL LEACHATES COLLECTED AFTER NATURAL RAINFALL FROM SOIL CORES[a] OF EUSTIS FINE SAND WHICH HAD BEEN TREATED WITH 2.5 CM OF SEEDED LIQUID SLUDGE (June 2, 1978 - August 24, 1978)

Dates	Cumulative rainfall (cm)	Soil core number	Cumulative leachate volume	Cumulative number of pore volumes[c] eluted
06-05-78 to 08-24-78	51.05	C1	1,544 (8.2)[e]	0.7
06-05-78 to 08-24-78	51.05	C2	1,135 (6.0)	0.5

Dates	Cumulative virus[d] eluted (total PFU)	Cumulative percent total virus applied	Range conductivity values of leachates collected (μmho/cm at 25°C)	Range pH values leachates collected
06-05-78 to 08-24-78	0	0	114 - 1,200	6.3 - 7.8
06-05-78 to 08-24-78	0	0	106 - 1,360	5.7 - 7.2

---

[a] 2.5 cm of lagooned sludge (sampled at W. Florida Agricultural Experiment Station, Jay; sludge had pH of 6.9 and solids content of 2.9%) seeded with a total of 7.8×10^8 PFU of poliovirus applied on top of 33 cm undisturbed soil cores of Eustis fine sand (15.5 cm inside diameter; contains Ap and A21 horizons). Seeded sludge was allowed to soak in and dry on top of soil 4 days before mixing with the top 2.5 cm of soil. Soil cores were exposed to natural conditions.

[b] Rainfall data collected at experimental site. Rainfall (cm) values represent total from the beginning of the experiment on 06-02-78.

[c] One pore volume for the large soil cores = 2,178 ml.

[d] Viruses in soil leachates were monitored by concentrating using membrane filtration, see "Materials and Methods" section.

[e] Values in parenthesis represent number of cm of cumulative leachate volume

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TABLE 7. ASSOCIATION BETWEEN 2 ENTEROVIRUSES AND LAGOONED SLUDGE[a] SOLIDS

Virus[b] used	Virus in unfractionated[c] sludge (total PFU)	Virus in sludge supernatant[d] (total PFU)	Viable unadsorbed[e] virus (% Vuv)	Sludge associated[f] virus (%)
Poliovirus type 1 (Sabin)	7.8×10^8	6.6×10^7	8.5	91.5[g]
	8.8×10^8	4.2×10^7	4.8	95.2[h]
Echovirus type 1 (Farouk)	2.9×10^6	2.3×10^6	78.3	21.7[h]

---

[a] The sludge used was lagooned sludge from the Jay Agricultural Research Center.

[b] Virus was added to sludge while magnetic stirring the suspension 1 hr.

[c] Sludge solids were not separated prior to assaying.

[d] Sludge was clarified by centrifugation at 20,842 x g for 30 min at 4°C; the supernatant was subsequently assayed.

[e] The Vuv% values were calculated on the corresponding unfractionated sludge assay.

[f] The "sludge associated virus (%)" values were estimated on the amount of virus recovered in the corresponding sludge supernatant.

[g] The sample of sludge used had a pH of 6.9 and a solids content of 2.9%. The virus seeded sludge was applied to soil columns.

[h] The sample of sludge used had a pH of 7.0 and a solids content of 7.0%. The virus seeded sludge was applied to soil columns.

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TABLE 8. SURVIVAL OF POLIOVIRUS TYPE 1 (SABIN) AND ECHOVIRUS TYPE 1 (FAROUK), UNDER NATURAL CONDITIONS, FOLLOWING SUSPENSION IN LIQUID SLUDGE AND SUBSEQUENT APPLICATION TO LARGE SOIL CORES OF A EUSTIS FINE SAND[a]

(October 11, 1978 - November 1, 1978)

					Number of viruses[c] (PFU/g dry weight of sludge or soil)			
					Echovirus		Poliovirus	
Date	Days after the beginning of experiment	Cumulative rainfall (cm)	Sludge solids (%, w/v)	Soil moisture (%, w/v)	Core 1	Core 2	Core 3	Core 4
Liquid sludge sample								
10-11-78[d]	0	0	7.0	-[e]	8.6×10^4	8.6×10^4	2.6×10^7	2.6×10^7
Dry sludge sample								
10-14-78	3	0	38.0	-	1.6×10^4	1.3×10^4	1.3×10^6	2.9×10^6
Sludge mixed with top 2.5 cm of soil on day 3								
Soil samples (top 2.5 cm)								
10-14-78	3	0	-	7.5	2.8×10^2	1.9×10^2	4.3×10^4	1.9×10^4
10-16-78	5	0	-	3.0	6.9×10^1	1.6×10^1	3.5×10^3	2.1×10^2
10-19-78	8	0	-	1.0	5.6×10^1	6.3×10^1	3.6×10^3	2.1×10^2
11-01-78	21	0.13	-	1.0	0	0	0	0

---

[a] 2.5 cm of lagooned sludge from Jay ARC (sludge had a pH of 7.0 and a solids content of 7.0%) seeded with 8.8 x 10 PFU of poliovirus or 2.9 x 10 PFU of echovirus applied on top of 33 cm undisturbed, large soil cores of Eustis fine sand. Seeded sludge was allowed to soak in and dry on top of the soil for 3 days before being mixed with the top 2.5 cm of soil.

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TABLE 8. Continued

[b] Rainfall data was collected at the experimental site.

[c] Virus monitoring in the drying sludge was undertaken using 0.05 M glycine, pH 11.5 as primary eluent, without further concentration of eluates. Virus monitoring of the soil was undertaken according to the isoelectric casein method.

[d] The liquid sludge was seeded with 2.6 x 10 PFU of poliovirus or 8.6 x 10 PFU of echovirus per gram dry weight of sludge. A large fraction of the polioviruses seeded (95.2%) were associated with the sludge solids after magnetic stirring for 1 hour and before application to the soil cores. Echovirus, on the other hand, was found mostly in the unadsorbed state (only 21.7% of the viruses were associated with the sludge solids). In addition to the large cores, the virus-seeded sludge was also applied (2.5 cm) to 2 undisturbed, small soil cores of Eustis fine sand (33 cm long and 5 cm inside diameter) for the purpose of studying transport of viruses only. One small soil core received poliovirus seeded sludge while the other received echovirus seeded sludge. Soil leachates from all cores were monitored for viruses.

[e] A dash means not done or not applicable

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TABLE 9. ANALYSES FOR THE PRESENCE OF POLIOVIRUS TYPE 2 (SABIN) AND ECHOVIRUS TYPE 1 (FAROUK) IN SOIL LEACHATES COLLECTED AFTER NATURAL RAINFALL FROM SOIL CORES WHICH HAD BEEN TREATED WITH 2.5 CM OF SEEDED LIQUID SLUDGE (October 11, 1978 - January 20, 1979)

Dates leachates collected	Cumulative rainfall[b] (cm)	Soil core number[c]	Cumulative leachate volume	Cumulative number of pore volumes eluted
12-01-78 to 01-21-79	24.95	SCI	400 (20.4)[f]	1.7
12-01-78 to 01-21-79	24.95	SC2	385 (19.6)	1.6
12-01-78 to 01-21-79	24.95	C1	750 (4.0)	0.3
12-01-78 to 01-21-79	24.95	C2	980 (4.9)	0.5
12-01-78 to 01-21-79	24.95	C3	920 (4.9)	0.4
12-28-78 to 01-21-79	24.95	C4	410 (2.2)	0.2

Dates leachates collected	Cumulative virus[e] eluted (total PFU)	Cumulative percent total virus applied	Range conductivity values of leachates collected (mho/cm at 25°C)	Range of pH values of leachates collected
12-01-78 to 01-21-79	0	0	140 - 625	5.8 - 7.0
12-01-78 to 01-21-79	0	0	135 - 710	6.3 - 7.0
12-01-78 to 01-21-79	0	0	375 - 800	6.3 - 6.8
12-01-78 to 01-21-79	0	0	190 - 975	6.1 - 6.3
12-01-78 to 01-21-79	0	0	280 - 1,200	5.9 - 6.9
12-28-78 to 01-21-79	0	0	560 - 875	6.0 - 6.9

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TABLE 9. Continued

[a] 2.5 cm of lagooned sludge (from Jay, ARC; the sludge had a pH of 7.0 and a solids content of 7.0%) seeded with a total of 8.8×10^8 (9.5×10^7 for the small core) PFU of poliovirus or 2.9×10^6 (3.1×10^5 for the small core) PFU of echovirus was applied on top of 33 cm undisturbed soil cores of Eustis fine sand (15.5 cm inside diameter for C1, C2, C3, and C4; 5 cm inside diameter for SC and SC2; contains Ap and A21 horizons). The seeded sludge was allowed to soak in and dry on top of the soil for 3 days before being mixed with the top 2.5 cm of soil. The soil cores were exposed to natural conditions.

[b] Rainfall data was collected at the experimental site. The cumulative rainfall (cm) values represent the total rainfall from the beginning of the experiment, October 11, 1978, to January 20, 1979.

[c] Echovirus was seeded in the sludge applied to small core 1, core 1 and core 2, core 3, and core 4.

[d] One pore volume for the large soil cores and the smallsoil cores was 2,178 ml and 234 ml, respectively.

[e] Viruses in soil leachates were monitored by concentration using membrane filtration.

[f] Values in parentheses represent the number of cm of cumulative leachate volume.

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TABLE 10. VIRUS ASSOCIATED WITH SLUDGE DISPOSAL AT THE KANAPAHA SITE

Sampling date	Sample	Enteroviruses (TCID 50)
December 1977	Wasted sludge	25.0
December 1977	Digested sludge[b]	1.2
December 1977, January 1978, and February 1978	Centrifuged sludge[c]	ND[d]
February 1978	Wasted sludge	11.0
February 1978	Digested sludge	0.3
December 1977	Soil[e]	ND
	Groundwater[f]	ND
January 1978	Soil	ND
	Groundwater	ND
February 1978	Soil	ND
	Groundwater	ND

---

[a] TCID per g (dry weight) of sludge or soil or 100 gallons of groundwater.

[b] From an aerobic digestor with a detention time of 90 days.

[c] The centrifuged sludge had undergone aerobic digestion for 180 days before conditioning and centrifugation.

[d] ND = not detected.

[e] 200 g composite soil samples.

[f] 100 gallons groundwater sampled at a 60 foot deep well (well location shown in Figure 2).

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TABLE 11. VIRUSES ASSOCIATED WITH DIGESTED SLUDGE ADDED TO THE SLUDGE LAGOON

Sludge source	Date Obtained	Type of Sludge Digestion Used	Viruses TCID$_{50}$/g
Montclair	02-17-78	Aerobic	260
Pensacola	02-17-78	Anaerobic	7
Montclair	12-06-78	Aerobic	41
Pensacola	12-06-78	Anaerobic	2
Montclair	02-12-79	Aerobic	14
Pensacola	02-12-79	Anaerobic	4

TABLE 12. ENTEROVIRUS NUMBERS IN THE OVERLYING WATER IN THE SLUDGE LAGOON

Date Obtained	Sludge Addition	Viruses TCID$_{50}$/l	Chlorophyll a mg/m^3
10-03-78	-[a]	< 0.5	2,370
11-06-79	-	< 0.5	1,508
12-14-78	+	9	387
01-11-79	+	55	172

[a] + indicates that sludge was being added to the lagoon at sampling times, - indicates that sludge was not being added to the lagoon at the sampling time.

TABLE 13. VIRUSES RECOVERED FROM LAGOON SLUDGE AND WATER SAMPLES AT JAY, FLORIDA

Sample	Sampling period	Total isolates identified	Polio-1	Polio-2	Polio-3	Echo-1	E4[1]	Echo-7	Echo-15	Cox. -B4[2]	Non-Typ.
Lagooned sludge	02-17-78 to 11-06-78	27	63	33	0	0	0	4	0	0	0
Digested sludge (Montclair and Pensacola)		32	81	9	9	0	0	0	0	0	0
Lagooned sludge	12-06-78 to 01-24-79	21	24	19	0	0	0	19	5	33	0
Water overlaying lagooned sludge		16	12	25	0	19	0	6	0	31	6
Digested sludge (Montclair and Pensacola)		10	20	0	30	20	0	20	0	10	0
Dried sludge	10-03-79 to 10-12-79	10	10	0	0	30	30	10	0	10	10
Total		116	46	17	5	7	3	8	1	12	2

[1] Echo-4

[2] Coxsackie

TABLE 14. SURVIVAL OF VIRUSES IN SLUDGE AFTER APPLICATION TO LAND AT JAY, FLORIDA

Date Obtained	Days after Spreading on land	% Water	$TCID_{50}/g$	
			Glycine Method	Urea-Lysine Method
10-03-79	0	91	1.4	4.6
10-04-79	1	39	< 0.01	0.16
10-05-79	2	40	0.72	0.10
10-08-79	5	ND	< 0.01	< 0.01
10-10-79	7	15	0.05	0.06
10-12-79	9	19	< 0.01	0.02

TABLE 15. ANALYSIS OF WELL WATER FOR THE PRESENCE OF ENTEROVIRUSES AT JAY, FLORIDA

Well no.	Site	Total Volume of Water sampled[a] (liters)	Viruses Detected
1	Northeast quadrant	1,100	0
3	Southwest corner (in direction of the groundwater flow)	1,100	0
9	East-central section	1,100	0
New lagoon	Approximately 10 ft from sludge lagoon	2,650	0
Total		5,950	0

[a] Six samples of 180 to 700 liters were obtained over a one year period.

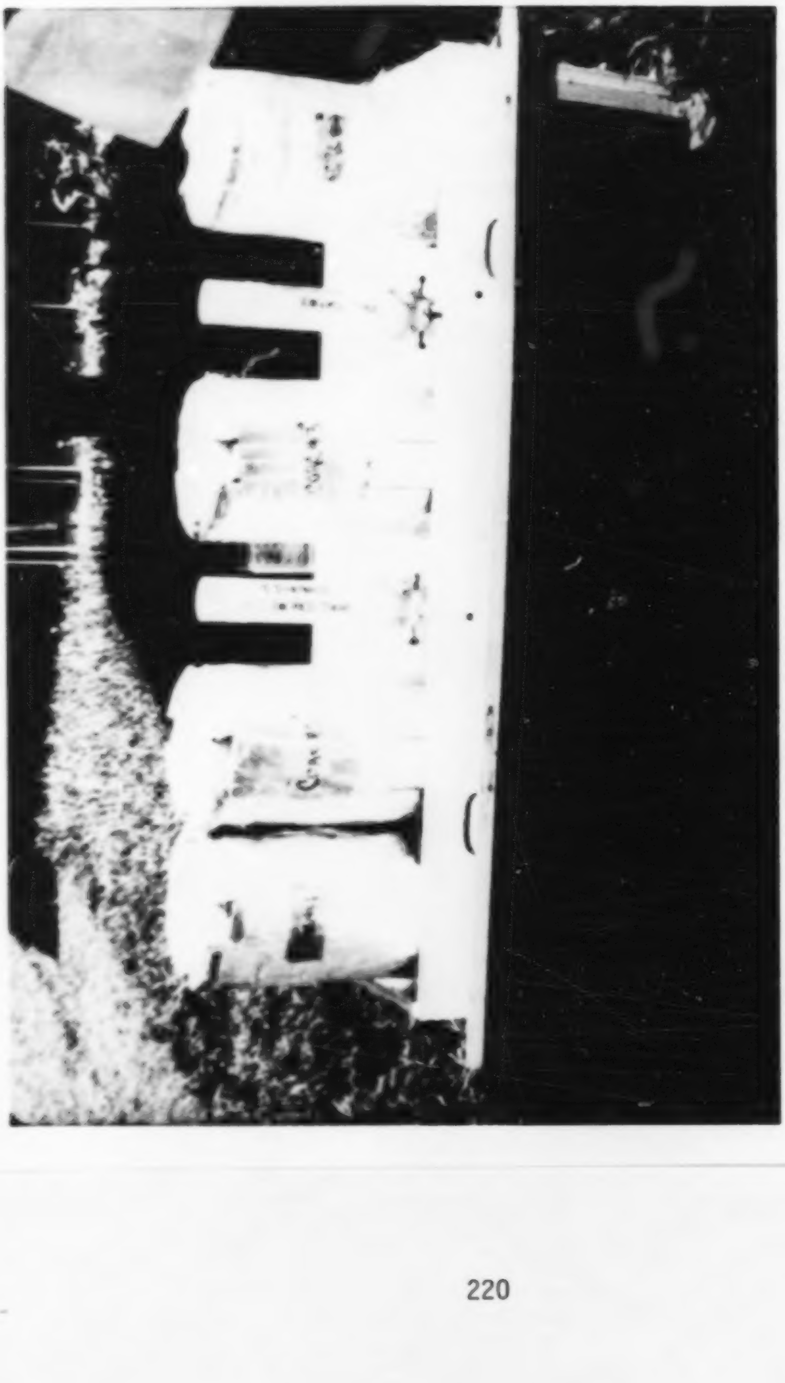


FIGURE 1. SOIL CORES

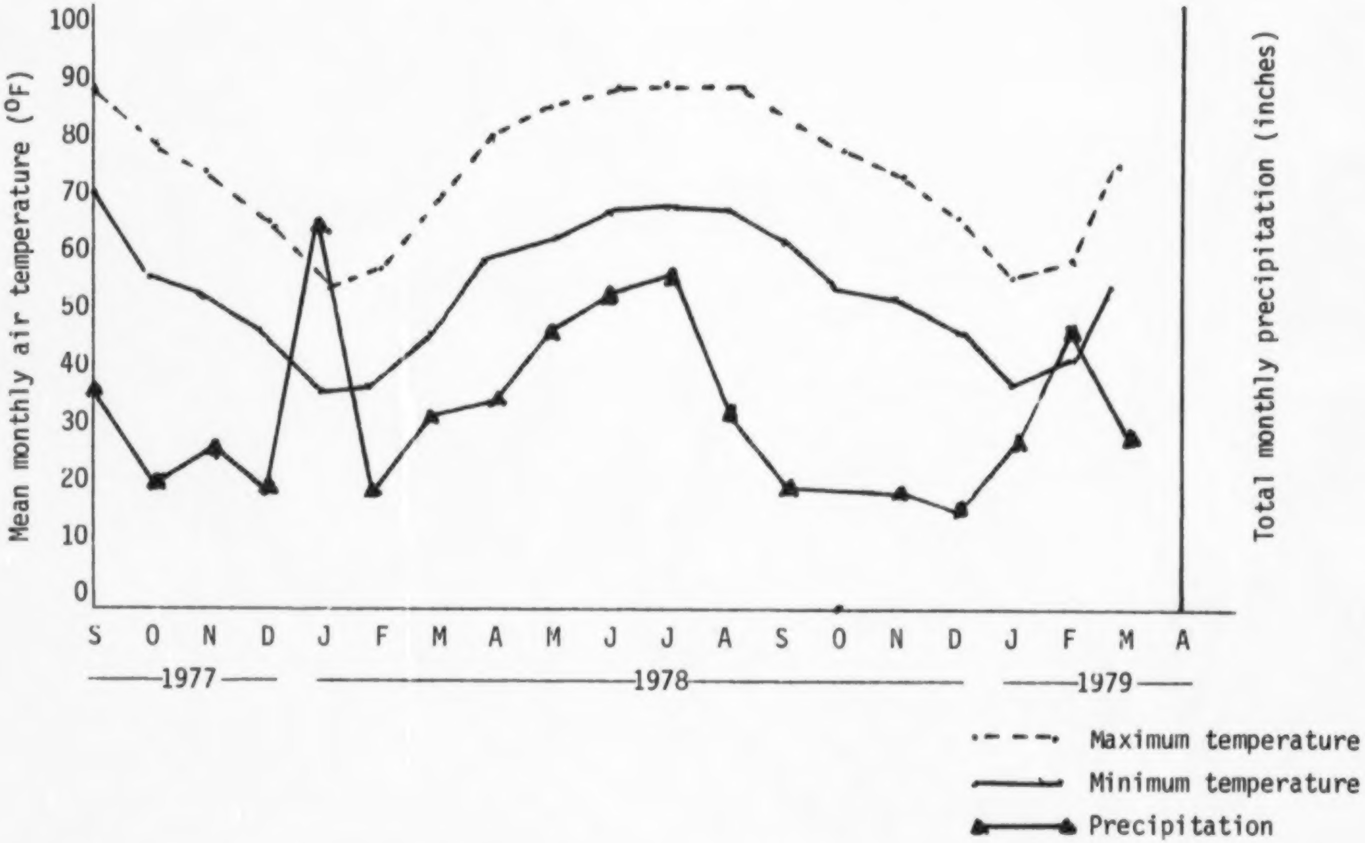


FIGURE 2. WEATHER DATA FOR THE WEST FLORIDA AGRICULTURAL EXPERIMENT STATION JAY, FL

WASTED SLUDGE

AEROBIC DIGESTION

Sludge treatment at the Main Street
Plant, Gainesville, Florida

(2 Digesters in series:
180 days detention time)

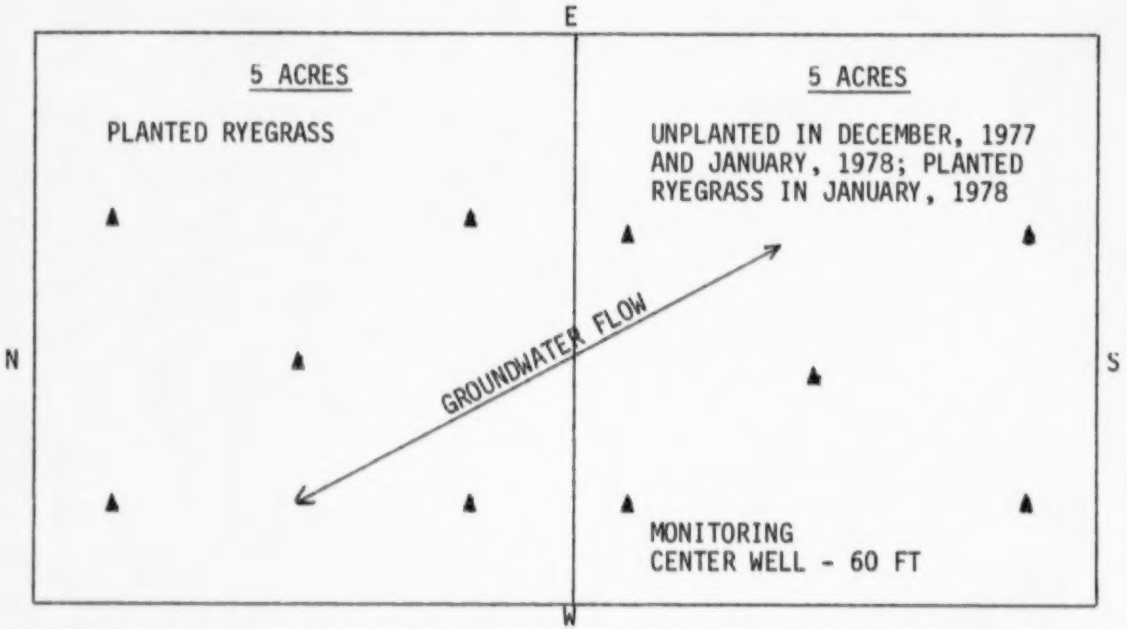
SLUDGE CONDITIONING

(Addition of Cationic Polymer)

CENTRIFUGATION

APPLICATION TO LAND AT THE KANAPAHA SITE
(10 acre plot)

FIGURE 3. SCHEME FOR SLUDGE DISPOSAL AT THE KANAPAHA SITE, GAINESVILLE, FLORIDA


E
N
S
W
5 ACRES
PLANTED RYEGRASS
5 ACRES
UNPLANTED IN DECEMBER, 1977 AND JANUARY, 1978; PLANTED RYEGRASS IN JANUARY, 1978
GROUNDWATER FLOW
MONITORING CENTER WELL - 60 FT

Δ Sampling points to obtain a 200 gram composite topsoil sample (40 grams per point) in each 5 acre plot per sampling date.

The soil found at this site belongs to the Lochloosa series.

FIGURE 4. DIAGRAM OF THE KANAPAHA SLUDGE DISPOSAL SITE

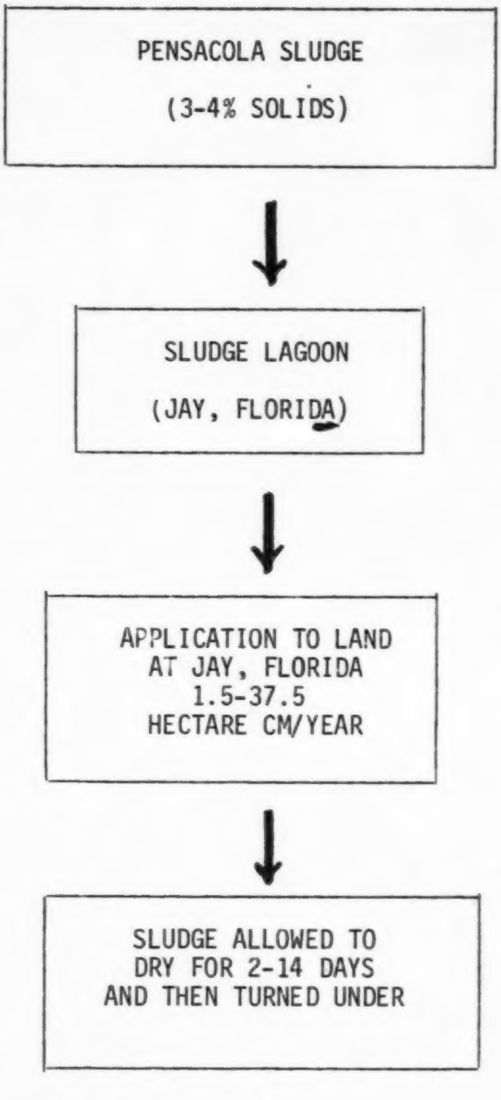

PENSACOLA SLUDGE
(3-4% SOLIDS)
SLUDGE LAGOON
(JAY, FLORIDA)
APPLICATION TO LAND
AT JAY, FLORIDA
1.5-37.5
HECTARE CM/YEAR
SLUDGE ALLOWED TO
DRY FOR 2-14 DAYS
AND THEN TURNED UNDER

FIGURE 5. SCHEME FOR SLUDGE DISPOSAL AT JAY SITE

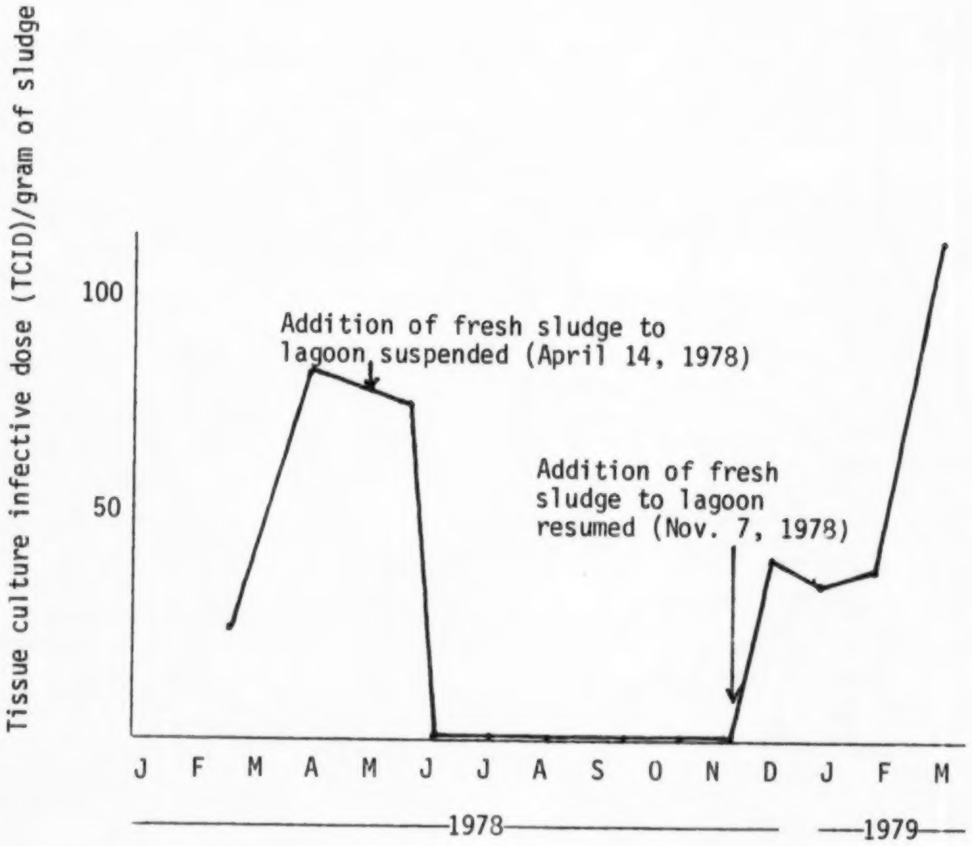

Tissue culture infective dose (TCID)/gram of sludge
100
50
Addition of fresh sludge to lagoon suspended (April 14, 1978)
Addition of fresh sludge to lagoon resumed (Nov. 7, 1978)
J
F
M
A
M
J
J
A
S
O
N
D
J
F
M
1978
1979

FIGURE 6.

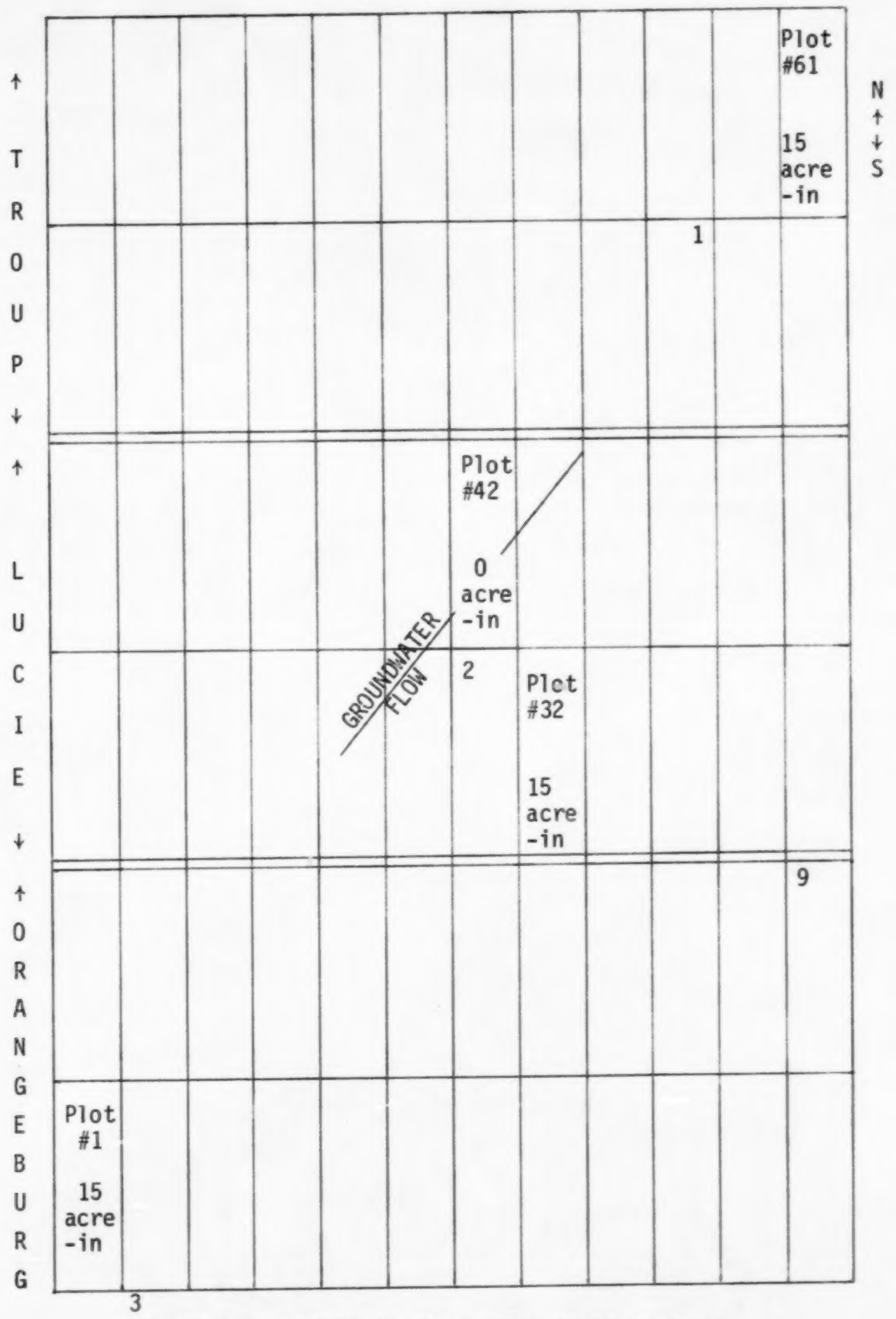

↑ TROUP ↓
↑ LUCIE ↓
↑ ORANGEBURG
N ↑ ↓ S
Plot #61
15 acre -in
1
Plot #42
0 acre -in
2
GROUNDWATER FLOW
Plot #32
15 acre -in
9
Plot #1
15 acre -in
3

FIGURE 7. DIAGRAM OF JAY SLUDGE DISPOSAL SITE

PESTICIDE RESIDUES IN FEED, SLUDGE, SOIL/SLUDGE, AND ANIMAL TISSUES

N. P. Thompson, O. Osuna, G. T. Edds

ABSTRACT

Samples of sludge, soil/sludge mixture, feed, and animal tissues (kidney, liver, fat, muscle) were analyzed for chlorinated hydrocarbon pesticide residues and also polychlorinated biphenyls. The analytical method is typical of that used for determination of persistent pesticide residues in environmental samples and includes Soxhlet extraction with petroleum ether; clean-up by gel permeation chromatography, florisil and silicic acid columns; and detection by electron capture gas chromatography. The sensitivity of the method is 0.01 ppm.

Results indicate that little if any chlorinated hydrocarbon residues were present in sludge used for research in this project. The available tables list the residues found in ppm in sludge, feed, and various animal tissues. It can be concluded that sludge as used in experiments associated with this project presents no hazard from the aspect of pesticide residues.

PESTICIDE ASSAYS - 1976-1980

	PCB	a-BHC	Lindane	Hepta-Chlor	Hepta-Chlor Epoxide	Aldrin	Dieldrin	Endrin	DDE	DDD	DDT	Chlor-dane
1976												
LiveOak Feed												
Control	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-
Control + 10% DFS	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-
Control + 20% DFS	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-
Jay Feed												
Control	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-
Corn	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-
Silage	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-
Concentration	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-
Soils (9)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-
Grass (6)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-
1977												
Poultry Feed												
50% "Corn"	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
100% "Corn"	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Control	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
LiveOak Feed												
Control	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Control + 10% DFS	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Control + 20% DFS	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
DFS	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Premix	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
DCS	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ration + 50%	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Control	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

All assays (4136) read as ppm; analyses by Varian Gas Chromatograph in Electron Capture Mode on 4% SE 30.6% OV 210. Calculations with Hewlett-Packard Integrator. Total assays performed - results negative to minimum detectable level. A very few sludge samples contained PCB's exceeding 5 ppm FDA guideline level.

OTHER ASSAYS - 1977, 1978. 1979

	PCB	a-BHC	Lindane	Hepta-Chlor	Hepta-Chlor Epoxide	Aldrin	Dieldrin	Endrin	DDE	DDD	DDT
Fat samples	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
LiveOak											
Control	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
ANS 10% DFS	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
ANS 20% DFS	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Jay											
Control	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
DPS:											
100 g/h/d	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
250 g/h/d	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
500 g/h/d	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
DCS:											
500 g/h/d	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Jay											
Fat	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Liver	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Kidney	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Brain	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Muscle	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
LiveOak											
Sows-Pigs:											
Kidney	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Liver	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Muscle	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Fat	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Total pesticide assays run (3 years) = 4136. Poultry - grain in ration - corn grown at Jay on 23 cm/hectare treated soil - 50% or 100% of corn in ration.

EPA Form 2350-6 (4-80)
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TYPING GUIDE SHEET

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